POLISH GYNECOLOGY

GINEKOLOGIA POLSKA

Edited since 1922

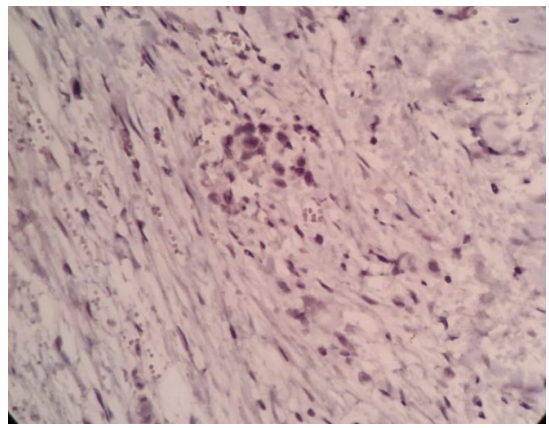
no **2**/vol **94**/**2023**

ORGAN POLSKIEGO TOWARZYSTWA GINEKOLOGÓW I POŁOŻNIKÓW
THE OFFICIAL JOURNAL OF THE POLISH SOCIETY OF GYNECOLOGISTS AND OBSTETRICIANS

IF: **1.216**, MNiSW: **40**

GINEKOLOGIA POLSKA 2023, VOL 94, NO 2, PAGES 87–168

ORIGINAL PAPERS



ISSN 0017–0011
e-ISSN 2543–6767

VIA MEDICA

VIA MEDICA | 30 LAT

OD 30 LAT
AKTYWNIE UCZESTNICZYMY W ROZWOJU NAUKI I EDUKACJI MEDYCZNEJ.

- W ciągu roku wydajemy ponad 800 publikacji
- Publikujemy blisko 50 czasopism
- Organizujemy ponad 300 konferencji i webinariów rocznie
- Udostępniamy około 3000 godzin filmów edukacyjnych
- Prowadzimy księgarnię medyczną Ikamed
- Obsługujemy 40 serwisów internetowych, oferujemy aplikacje mobilne

www.viamedica.pl

@ViaMedicaMedicalInformation

POLISH GYNECOLOGY

GINEKOLOGIA POLSKA

ORGAN POLSKIEGO TOWARZYSTWA GINEKOLOGÓW I POŁOŻNIKÓW
THE OFFICIAL JOURNAL OF THE POLISH SOCIETY OF GYNECOLOGISTS AND OBSTETRICIANS

ISSN 0017–0011
e-ISSN 2543–6767

Ginekologia Polska is published monthly, twelve volumes a year, by VM Media Group sp z o.o.
73 Świętokrzyska St, 80–180 Gdańsk, Poland, phone: (+48 58) 320 94 94, fax: (+48 58) 320 94 60,
e-mail: redakcja@viamedica.pl, marketing@viamedica.pl, http://www.viamedica.pl

Editorial office address: Woman's Health Institute, School of Health Sciences, Medical University of Silesia in Katowice, 12 Medyków St, 40–752 Katowice, e-mail: ginpol@viamedica.pl

Indexed in: CrossRef, DOAJ, Index Copernicus, Polish Ministry of Education and Science (40), POL-Index, Polish Medical Bibliography, PubMed, Science Citation Index Expanded (1.216), Scimago Journal Rank, Scopus, Ulrich's Periodicals Directory

Advertising. For details on media opportunities within this journal please contact the advertising sales department,
73 Świętokrzyska St, 80–180 Gdańsk, Poland, phone: (+48 58) 320 94 94, e-mail: marketing@viamedica.pl

Subscription for 2023. Printed institutional subscription — 12 issues for 360 EUR. Printed individual subscription — 12 issues for 180 EUR.
More details at: https://journals.viamedica.pl/ginekologia_polska/user/subscriptions

22-6120.002.001

EUROPEAN CONGRESS OF OBSTETRICS AND GYNAECOLOGY

28th EBCOG CONGRESS

KRAKOW · 18th–20th MAY · 2023

Women's Health in Europe

facing up to the challenges

Dear Colleagues and Friends,

The European family of obstetricians and gynaecologists (EBCOG) together with the Polish Society of Gynecologists and Obstetricians will join for an unforgettable meeting in Krakow, Poland.

The most respected European scientists have agreed without hesitation to come to Krakow for their keynote lecture. This congress will offer you insight in novel techniques, debates on equal opportunities, accreditation of centers, staff training, Women's Rights and influence of Climate changes on our well being, prenatal diagnostics, gynecological oncology, obstetrics and much more.

Join us in beautiful Krakow the old royal capital of Poland.

Prof. Basil C. Tarlatzis · Dr. Rudi Campo · Prof. Piotr Sieroszewski · Prof. Sebastian Kwiatkowski

For the first time in Poland! European Congress

- Climate change and health issues
- Sexual and Reproductive Health
- Obesity, lifestyle, and women's health
- Impact of war on maternal health
- Modern gynaecological surgery
- Vaginal breech delivery

Keynote Speakers:

- Jeanne Conry
- Kypros Nicolaides
- Rudi Campo
- Diogo Ayres-de-Campos
- Arnaud Wattiez

ICE Krakow Congress Centre

More information on our website

www.ebcog2023.com

POLISH GYNECOLOGY

GINEKOLOGIA POLSKA

ORGAN POLSKIEGO TOWARZYSTWA GINEKOLOGÓW I POŁOŻNIKÓW
THE OFFICIAL JOURNAL OF THE POLISH SOCIETY OF GYNECOLOGISTS AND OBSTETRICIANS

CONTENTS

VIA MEDICA

EDITORIAL

Ginekologia Polska
2023, vol. 94, no. 2, 87–88

ISSN 0017–0011, e-ISSN 2543–6767

DOI 10.5603/GP.a2023.0023

Adolescence pregnancy as a challenge of modern perinatology

Jakub Staniczek, Rafal Stojko, Agnieszka Drosdzol-Cop

Chair and Department of Gynecology, Obstetrics and Gynecological Oncology, Faculty of Health Science in Katowice, Medical University of Silesia in Katowice, Poland

Nearly 16 million teenage girls aged 15–19 and two million girls under 15 becoming pregnant each year [1]. Due to possible problems and complications adolescent pregnancies should be treated as a high-risk pregnancy. The most common obstetric pathologies in young mothers, includes preeclampsia, hypertension, diabetes mellitus, fetal growth restriction and premature birth, as a consequence of immaturity, especially uterine immaturity. The risk of preeclampsia and eclampsia among this group is almost two times higher than among 20 — to 24-year-old women. Preterm labor occurs with a frequency of about 15%. Additionally, young mothers have a significantly increased risk of having extremely premature babies and newborns with extremely low birth weight [2–4].

An important aspect is ultrasound diagnostics in adolescence pregnancy. Attention should be paid to the frequent occurrence of fetal anomalies in this group of patients. Prenatal diagnosis should also be focused on the assessment of the risk of preterm delivery, fetal growth restriction and pre-eclampsia, both with the use of modern markers of angiogenesis. Non-chromosomal anomalies are common in teenage pregnancies and are estimated to occur in 26.5/1000 children. Statistically, abdominal wall defects, anomalies of the gastrointestinal tract, central nervous system and heart are more frequent than in older patients. Studies have shown that younger women have lower awareness of folic acid supplementation, resulting in a higher incidence of spina bifida/myelomeningocele in this group [5, 6].

In recent years, we have seen much earlier sexual initiation. In a group of 15-year-olds, it showed that 9.2% of 15-year-old girls had sexual initiation. Among all sexually active 15-year-olds, 27% did not use any method of contraception during intercourse. It is worth emphasizing that, sexually transmitted infections and bacterial vaginosis are common in this group, that's why routine screening should be done. Early sexual initiation is also associated with a high-risk non-immune hydrops fetalis corresponding with maternal infection.

It is important to remember to promote adequate supplementation in pregnant minors. We must bear in mind the increased iron, calcium and vitamin D requirements of adolescent girls during pregnancy, both due to pregnancy and the ongoing skeletal ossification process. Proper nutrition is also a strategy to reduce anemia and low birth weight and to optimize weight gain in pregnancy. Young pregnant women are significantly more likely to use drugs and alcohol than their non-pregnant peers. This contributes to a higher incidence of Fetal Alcohol Syndrome (FAS) in newborns [5, 7, 8].

The literature data shows differences in the number of vaginal deliveries and cesarean sections in the group of teenagers. Adolescent pregnancy is not an indication for caesarean section, unless there are clear indications for surgery. The attention should be paid to the structure and dimensions of the bony pelvis. A higher rate of cesarean sections in adolescents < 15 years of age which may be due to their biological immaturity [9].

Adolescent pregnancy is a challenge not only from the medical side but also from the social and legal point of view. The support of the medical staff and a psychologist may be crucial not only for the proper development of pregnancy, but also for preparing a young girl to take up the challenge of parenthood. Young mothers often experience exclusion and 25% of them experience postpartum depression [10].

To conclude, pregnancy in teenagers require exceptional skills, knowledge and it is a challenge of modern perinatology, so we invite you to the next issue of "Ginekologia Polska", where you will find the PTGiP Recommendations on the management of adolescence pregnancy.

Corresponding author:
Agnieszka Drosdzol-Cop
Chair and Department of Gynecology, Obstetrics and Gynecological Oncology, Faculty of Health Science in Katowice, Medical University of Silesia in Katowice, Poland
e-mail: cor111@poczta.onet.pl

Received: 20.02.2023 Accepted: 22.02.2023 Early publication date: 24.02.2023

Conflict of interest

All authors declare no conflict of interest.

REFERENCES

1. Girlhood, Not Motherhood: Preventing Adolescent Pregnancy Published by the United Nations Population Fund UNFPA, New York 2015 ISBN: 978-0-89714-986-0. https://www.unfpa.org/sites/default/files/pub-pdf/Girlhood_not_motherhood_final_web.pdf (20.02.2023).
2. Doroftei B, Ilie O, Maftei R, et al. The pregnancy rate among Romanian adolescents: an eleven years (2009-–2020) observational, retrospective study from a single center. Ginekologia Polska. 2022; 93(1): 42–46, doi: 10.5603/gp.a2021.0132.
3. Guimarães A, Bettiol H, Souza L, et al. Is adolescent pregnancy a risk factor for low birth weight? Revista de Saúde Pública. 2013; 47(1): 11–19, doi: 10.1590/s0034-89102013000100003.
4. Macedo T, Montagna E, Trevisan C, et al. Prevalence of preeclampsia and eclampsia in adolescent pregnancy: A systematic review and meta-analysis of 291,247 adolescents worldwide since 1969. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2020; 248: 177–186, doi: 10.1016/j.ejogrb.2020.03.043.
5. Zimmer M, Sieroszewski P, Oszukowski P, et al. Polish Society of Gynecologists and Obstetricians recommendations on supplementation in pregnancy. Ginekologia Polska. 2020; 91(10): 644–653, doi: 10.5603/gp.2020.0159.
6. Sieroszewski P, Haus O, Zimmer M, et al. Recommendations for prenatal diagnostics of the Polish Society of Gynaecologists and Obstetricians and the Polish Society of Human Genetics. Ginekologia Polska. 2022; 93(5): 427–437, doi: 10.5603/gp.a2021.0255, indexed in Pubmed: 35315029.
7. ALLARD-HENDREN R. Alcohol Use and Adolescent Pregnancy. MCN, The American Journal of Maternal/Child Nursing. 2000; 25(3): 159–162, doi: 10.1097/00005721-200005000-00010.
8. Fleming N, O'Driscoll T, Becker G, et al. Adolescent Pregnancy Guidelines. Journal of Obstetrics and Gynaecology Canada. 2015; 37(8): 740–756, doi: 10.1016/s1701-2163(15)30180-8.
9. Karataşlı V, Kanmaz AG, İnan AH, et al. Maternal and neonatal outcomes of adolescent pregnancy. J Gynecol Obstet Hum Reprod. 2019; 48(5): 347–350, doi: 10.1016/j.jogoh.2019.02.011, indexed in Pubmed: 30794955.
10. Dominiak M, Antosik-Wojcinska AZ, Baron M, et al. Recommendations for the prevention and treatment of postpartum depression. Ginekol Pol. 2021; 92(2): 153–164, doi: 10.5603/GP.a2020.0141, indexed in Pubmed: 33448014.

VIA MEDICA

ORIGINAL PAPER / GYNECOLOGY

Ginekologia Polska
2023, vol. 94, no. 2, 89–94

ISSN 0017–0011, e-ISSN 2543–6767

DOI 10.5603/GP.a2022.0097

Effects of polyamine synthesis enzymes on angiogenesis and apoptosis during endometriosis

Caglar Yildiz[1], Veysel Kenan Celik[1], Begum Kurt[1], Serkan Kapancık[2], Hasan Kılıcgun[3]

[1]Cumhuriyet University, Turkey
[2]Okan University Faculty of Medicine Department of Biochemistry, Istanbul, Turkey
[3]Erzincan Binali Yildirim University, Erzincan, Turkey

ABSTRACT

Objectives: Since we assumed that endometriosis is a benign cell division disorder, our study was conducted to investigate the effects of the relationships between polyamine synthesis and angiogenesis in the formation of endometriosis.

Material and methods: Thirty-five patients with endometriosis and 35 healthy female women were included in the study. The patient and the control groups were compared regarding the blood levels of agmatine, argininecarboxylase (ADC), ornithinecarboxylase (ODC), agmatinase, arginase, ornithine, and the vascular endothelial growth factor (VEGF).

Results: There is a statistically significant difference between the patient and the control groups regarding the agmatinase, arginase and VEGF levels (higher in the patient group) ($p < 0.05$). There is no statistically significant difference between the patient and the control groups regarding the ODC, ornithine and the ADC levels ($p > 0.05$). There is a statistically significant difference between the patient and the control groups regarding the agmatine levels (higher in the control group) ($p < 0.05$).

Conclusions: The increase in the serum levels of polyamine synthesis enzymes may contribute to the formation of endometriosis. It is anticipated that the study of the relationship between enzymes and molecules in the polyamine synthesis pathway and angiogenesis in patients with endometriosis will contribute to the literature.

Key words: angiogenesis; apoptosis; endometriosis; polyamine

Ginekologia Polska 2023; 94, 2: 89–94

INTRODUCTION

Endometriosis is a chronic, benign, often painful disease seen in 8–18% of young women. The disease causes severe physical and mental problems. Endometriosis is a disease that develops due to the formation of a tissue similar to the uterine mucosa in the outer part of the uterus in the sub-abdominal region, and its spread to various organs (such as the uterus, peritoneum, oviducts, ovaries, bladder, and intestines). It has been reported that the number of polyamines, which is also indicated to be associated with the physiopathology of endometriosis, increases in body fluids. In this study, the levels of agmatine, which is essential in the production of major polyamines, the key enzymes in the synthesis of polyamines [arginase, argininecarboxylase (ADC), ornithinecarboxylase (ODC), agmatinase], ornithine, which is an intermediate product, and the vascular endothelial growth factor (VEGF) levels were investigated in cases who has endometriosis.

Polyamines are organic cations found naturally in microorganism, animals, and plants. Putrescine, the precursor of major polyamines, is synthesized from arginine in two different ways through a total of four enzymes (arginase, ADC, ODC and agmatinase). The ability of polyamines to work is related to the electrical charge they carry. Although first detected in semen, polyamines are found in varying amounts in many cell types. They are mostly observed in cells with the highest amount of rapid turn-over [1, 2].Polyamines are essential for normal cell and tissue functions such as development, growth and tissue repair. They have roles in cell proliferation, cell growth, production of proteins and nucleic acids, repair of extracellular matrix, cell adhesion and signal transduction processes [1, 3, 4]. Polyamines are metabolized by aminoxidases and acetyltransferases [2, 5]. They most likely exert this effect through the nuclear phosphoprotein p53. It was observed that the decrease

Corresponding author:
Caglar Yildiz
Cumhuriyet University, Turkey
e-mail: dr_caglaryildiz@yahoo.com

Received: 14.03.2022 Accepted: 27.08.2022 Early publication date: 10.10.2022

in the amount of polyamine increased p53 gene expression and suppressed cell growth [2, 6–10]. Polyamines stimulate NF-KB binding to the specific response elements on DNA [11]. Polyamines reduce macrophage activation, via NO metabolism. [12].

In this study, the relationships between polyamine synthesis and angiogenesis in the formation of endometriosis were investigated.

MATERIAL AND METHODS

The patients and control groups

Between 01.06.2016–01.06. 2017, thirty-five patients, with endometriosis were included in the study. There was no age restriction for inclusion to the study. Our control group consisted of 35 healthy female hospital personnel without any systemic disease (diabetes or hypertension). In this study, patients who underwent surgical operation for benign reasons in gynecology clinic and diagnosed with endometriosis pathological examination were selected as the study group. As the control group, patients who underwent surgery for benign reasons, who had no chronic disease and no endometriosis disease were selected. Local ethics committee approved this study (Ethics Committee no: 2015-11/40; dates: 24.11.2015), and consent of the participants was obtained for the study.

Collection of samples

Ten mL of whole blood were obtained from the participants, centrifuged at 4000 rpm for 15 minutes, and the serum obtained was aliquoted and stored at -80 °C until analysis. The measurements were repeated 3 times and the mean values of the measurements were used.

Determination of arginase activity

We added 9.9 mL of 2.5 mM MnCl2 to 0.1 mL of hemolyzate and left at 55°C for 10 min preincubation. 0.4 mL of 50 mM arginine solution and 0.4 mL of 100 mM carbonate buffer (pH 9.7) were added to the test and zero-time blank tubes. We placed 1 mL of distilled water in the blank tube and 1 mL of urea standard of 0.1 µmol/mL in the standard tube was placed in the blank tube also. In the zero-time tube, 3 mL of the acid mixture (0.12 M FeCl3 and 20% (v/v) H2SO4 mixture in 56.7% H3PO4) was added followed by addition of 0.2 mL of enzyme source and the mixture was vortexed. We placed 3 mL of acid mixture in standard and blind tubes. To reach the same temperature, the tubes and the enzyme source were allowed to stand in the 37°C metabolic water bath for three minutes. Then 0.2 mL of enzyme source was added in the test tubes, vortexed and left in the metabolic water bath for 15 min at 37°C. At the end of this time, the reaction was stopped by adding 3 mL of acid mixture to the test tubes.We added 2 mL of color separator (0.0036 M thiosemicarbazide + 0.0617 M diacetylmonoxime) to all of the tubes and vortexed. The tips of the tubes were closed and kept in a boiling water bath for 10 minutes and the absorbances were read against the blood at 520 nm.

In the calculation; a net absorbance was obtained by subtracting the zero time blank absorbance from the absorbance of each test tube. Thus, the endogenous urine absorbance at the source of the enzyme was excluded from the calculation. A common factor was found from standard absorbance, standard urea amount and dilution coefficients. Factor calculation was done as follows:

$$\text{Factor} = (0.1\ \mu\text{mol urea /mL}) \times 10 \times 5 \times 4$$

The net sample absorbance was multiplied by the factor in the calculation of the enzyme activity. Enzyme activities were found in µmol urea/mL/hour (absorbance of 0.1 µmol urea/mL).

Ornithine assay

Initially, the Serum sample was mixed with 1/1 water and then with 1/1 TCA 10%. The supernatant was removed after centrifugation for five minutes at 3000 rpm. Then 1 mL of supernatant, 1 mL of distilled water and 1 mL of 0.18 µmol/mL ornithine solution were added to the test tube, blind tube and standard tube respectively. Next, 2.5 mL of glacial acetic acid and 0.25 mL of ninhydrin were added to the tubes. The prepared tubes were vortexed and mixed in a boiling water bath for 30 minutes. After the water bath, the tubes were allowed to cool immediately and the absorbances were measured at 515 nm with a spectrophotometer.

The ornithine level was calculated as follows:

$$\text{Ornithine (µmol/L)} = \frac{\text{Test Absorbance} \times 2 \times \text{Standard Concentration}}{\text{Standard Ornithine Absorbance Value}}$$

Determination of agmatine levels in serum by HPLC method

Sample preparation and derivatization

Each of the 2 mL serum samples obtained from the patients and the controls were separated in two different tubes of 1 mL. The first tube was treated with 100µl of deionized water. The same amount of agmatine standard (2 µM concentration) was placed in the second tube. Before the plasma sample in each tube was centrifuged at 1000 × g for 15 minutes at 4˚C, deproteinization was performed with 700 µL of 1M perchloric acid + 0.1 M hydrochloric acid solution and kept in ice for one hour. 1.5 mL supernatant samples were neutralized with 5M NaOH and mixed with 750 µ volume of derivatization reagent. Preparation of o-phthaaldehyde (OPA) and 2-mercaptoethanol (ME) derivatization reagent: 50mg of OPA was dissolved in 1 mL of methanol and ME solution (53 µL ME was mixed into 9 mL volume of 3% KOH + %3H2BO4 solution) was added.

Extraction, concentration and measurement of serum agmatine

Due to the materials in the serum that may interact with OPA, the obtained samples were firstly extracted with the C18 cartridge. and then injected into the HPLC system. (Chromoband, Machery e Nagel, Duren, Germany). First, this cartridge was washed with water and methanol. 2.25 mL derivatized samples were applied to the cartridge. After this, the cartridge is filled with 3 mL of water, methanol and acetonitrile [1/1/1 (v/v/v)], pH13 adjusted with NaOH) and 100 mL of water, methanol and acetonitrile [1/3/5 (v/v/v)], pH: 3.5, adjusted with acetic acid). Agmatine was eluted with a second 100mL of water, acetonitrile and methanol [1/3/5 (v/v/v)], pH3.5, adjusted with acetic acid). We quickly injected 20 µL of the eluate into HPLC. Agmatine levels were determined using the HPLC system. The HPLC system had a quaternary pump and a fluorescence detector. The excitation wavelength of the fluorescent detector was set to 350 nm and the emission wavelength to 450 nm. Chromatography column C18, Nucleosil 250 mm _4 mm i.d. (Hichrom, Berkshire, UK). The column temperature was set to 400C. The mobile phase was formed from a mixture of 10mM KH_2PO_4 (46%), acetonitrile (34%), methanol (20%) and 4.5 mM octyl sulfate sodium salt (pH = 7). The flow rate was set to be 1 mL/min. Agmatine amounts were measured by comparing plasma sample chromatograms and standard chromatograms [13].

VEGF enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay was used to analyze serum VEGF (Cat. No: E0050Hu). The concentration range of the VEGF standard solution was 3–900 ng/L. Added 40 µL sample 10 µL Human VEGFA antibody, and then 50 µL streptavidin-HRP to wells. Plates were incubated at 37 °C for 60 min in the dark. Following incubation, the plates were run through the buffer solution five times and combined with 50 µl of substrate solution A and 50 µL of substrate solution B. Incubation of the plates was continued for 10 minutes in the dark at 37°C. The reaction was terminated with 50 µL of stop solution, after 10 µL the absorbance value was measured at 450 nm.

Statistical analysis

In this study, having $\alpha = 0.05$; $\beta = 0.20$ and $(1-\beta) = 0.80$, the number of individuals constituting the patient and the control group was determined to be 35 and the strength of the test was found to be $p = 0.80555$. The data obtained in the study were loaded on the SPSS (Ver: 22.0) program and evaluated from the data; when the parametric test assumptions were fulfilled (Kolmogorov-Smirnov), the significance test of the difference between the two means was used, and the Mann Whitney U test was used when the parametric test assumptions were not fulfilled. Regression and the correlation analyses were applied and the error level was taken as 0.05.

RESULTS

The descriptive statistics of endometriosis patients (Tab. 1). Dysmenorrhea and dyspareunia symptoms were more common in the patient group than in the control group. Infertility was detected more frequently in the study group than in the control group. In addition, chronic pelvic pain was seen more frequently in study group, the difference was statistically significant (Tab. 1).

Since we hypothesize that endometriosis is a benign cell division disorder, agmatinase, ODC, ADC, agmatine,

Table 1. The descriptive statistics of endometriosis patient

		Endometriosis	Control	p value
Ages	[years] mean ± SD	38.12 ± 9.12	35.82 ± 8.25	
		Endometriosis (n, %)	**Control (n, %)**	**p value**
Family history of endometriosis	No family history	29 (82.9)	32(8.5)	
	First degree relative	6 (17.1)	3(91.5)	
Infertility	Yes	11 (31.5)	2 (5.7)	
	No	24 (68.5)	33 (94.3)	
Dysmenorrhea symptoms	Yes	27 (77.2)	8 (22.9)	
	No	8 (22.8)	27 (77.1)	
Dyspareunia symptoms	Yes	22 (62.9)	5 (14.3)	
	No	13 (37.1)	30 (85.7)	
Cronic pelvic pain	Yes	20 (57.2)	12 (34.3)	
	No	15 (42.8)	23 (65.7)	

n — number, ($p < 0.05$), compared to control group

Table 2. The results of the Mann Whitney test comparing the levels of agmatinase, ornithinecarboxylase, argininecarboxylase, agmatine, vascular endothelial growth factor, ornithine, arginase in the patient and the control groups

	n	Mean	Standard deviation	$\overline{X}_{sira}$	Σ_{sira}	U	z	p
Patient, agmatinase	36	356.92	352.74	46.42	1671.00	291.000	–4.021	0.000
Control agmatinase	36	110.39	73.06	26.58	957.00			
Patient ODC	36	1876.57	2094.92	40.90	1472.50	629.000	–0.214	0.831
Control ODC	36	826.82	527.85	32.10	1155.50			
Patient ADC	36	1006.56	1051.89	41.29	1486.50	475.500	–1.943	0.052
Control ADC	36	519.56	321.15	31.71	1141.50			
Patient agmatine	36	6.58	4.16	29.64	1067.00	401.00	–2.782	0.005
Control agmatine	36	10.85	6.72	43.36	1561.00			
Patient VEGF	36	181.07	174.05	41.89	1508.00	454.000	–2.815	0.029
Control VEGF	36	105.41	111.56	31.11	1120.00			
Patient ornithine	36	0.09	0.01	39.26	1413.50	548.500	–1.160	0.246
Control ornithine	36	0.08	0.01	33.74	1214.50			
Patient arginase	36	13.59	6.86	45.79	1648.50	313.500	–3.768	0.000
Control arginase	36	8.02	5.10	27.21	979.50			

The cut-off value of $p < 0.05$ was used to interpret the analysis results at the 95% confidence level. $p < 0.001$ is used to show that results are less than 0.1% common; ODC — ornithinecarboxylase; ADC — argininecarboxylase; VEGF — vascular endothelial growth factor

VEGF, ornithine, arginase agmatinase levels were examined in the patients with endometriosis and the obtained values were compared with the control group (Tab. 2). There is a statistically significant difference between the patient and the control groups regarding the agmatinase levels (higher in the patient group) ($p < 0.01$). When the ODC levels were compared, no statistically significant difference was found between the study groups. ($p > 0.05$). There is no statistically significant difference between study groups regarding the ADC levels ($p > 0.05$). There is a statistically significant difference between the patient and the control groups regarding the agmatine levels (higher in the control group) ($p < 0.05$). There is a statistically significant difference between the patient and the control groups regarding the VEGF levels (higher in the patient group) ($p < 0.01$). There is no statistically significant difference between study groups regarding the ornithine levels ($p > 0.05$). There is a statistically significant difference between the study groups regarding the arginase levels (higher in the patient group) ($p < 0.01$).

The patient and the control groups were compared regarding the levels of agmatine, ADC, ODC, agmatinase, arginase, ornithine, and the VEGF. Although the patient group's ODC and ADC levels are increased by approximately 200% compared to the control group, there was no statistically significant difference between the two groups in terms of ornithine levels ($p > 0.05$). Agmatinase, arginase, and VEGF levels were higher in the patient group than those in the control group, and the difference was statistically significant ($p < 0.05$). Agmatine levels were lower in the patient group compared to the control group, and the difference was statistically significant ($p < 0.05$).

As shown in Table 1, it is observed that arginase activity, which is the first enzyme of the polyamine synthesis pathway, is increased in the patient group compared to the control group. This increase is statistically significant ($p < 0.05$).

DISCUSSION

Endometriosis is a common gynecological disease of benign, hormonal origin. Pelvic pain, dysmenorrhea, dyspareunia, and infertility are common clinical findings of endometriosis [14]. Histologically, it is accepted that benign endometriotic lesions occur as a result of genetic errors that can also lead to malignant transformations. Studies have detected loss of heterozygosity and the presence of mutations in tumor suppressor genes. This disease shows high genetic instability that plays a role in the cellular phenotypic changes involved in cancer progression [15].

In the studies conducted in different cancer types including lung [16], colorectal [17], prostate [18], pancreatic [19], skin [20] and stomach [21] cancers, increased arginase activity has been detected and it has been directly associated with cancer. Assuming that endometriosis is a benign cell division disorder disease, our findings support the studies showing increasing arginase activity. To the best of our knowledge, arginase activity has not been studied in patients with endometriosis to date, this makes our results meaningful.

Increased arginase activity will increase ornithine levels. However, no increase in ornithine levels was observed in our study. The reason for this finding is the increase of ODC levels by 200% compared to the control group, although it

was not statistically significant. Increased ODC levels convert ornithine, which is formed twice as fast, to putrescine. The studies reporting that ornithine levels do not change in many cancers such as breast cancer [22] and colorectal cancer is in line with our results. On the other hand, the fact that ODC levels are increased in prostate tissue of patients with prostate cancer [23], in breast cancer [24] and in esophageal cancer [25], supports our data.

ADC, which is the first enzyme in the alternative second pathway of the synthesis pathway, was observed to have doubled in the sera of the patients with endometriosis when compared to the control group, but it was not statistically significant (Tab. 2). Although this increase in ADC seems to be in the direction of agmatine formation, in patients with endometriosis, agmatine levels measured by HPLC showed a decrease of approximately 40% when compared to the control group, and it was statistically quite significant ($p < 0.05$). The reason why the increase in ADC levels could not turn in favor of agmatine is the 300% increase in agmatinase enzyme levels that use agmatine as a substrate. Since this increase in agmatinase levels will cause the formation of putrescine, it is significant ($p < 0.001$). Decreased amount of agmatine cannot control ODC and decrease its proliferation rate [26, 27]. The increase in the amount of enzymes in the polyamine synthesis pathways (ornithine and agmatine pathway) caused an increase in putrescine levels. On the other hand, an increase of 200% in VEGF levels, which is an indicator of angiogenesis, shows that vascularization with proliferation is continued [28]. This statistically significant increase indicates that the apoptosis process has started. According to the study of Vodolazkaia et al. [29]; similar to our findings, women with endometriosis had a significantly higher plasma VEGF concentration compared to controls. VEGF is necessary for the growth of eutopic and ectopic endometriotic tissues [30].

CONCLUSIONS

In conclusion, the increase in the serum levels of polyamine synthesis enzymes may be contributing to the formation of endometriosis. The fact that the decreased agmatine levels do not have an inhibitory effect on ODC, the regulatory enzyme in polyamine synthesis, shifts the synthesis pathway to the direction of the synthesis of major polyamines. Thus, the increase in proliferation causes the continuation of angiogenesis. This is confirmed by the demonstration of the increase in VEGF levels. Revealing the relationship between the enzymes and the molecules in the polyamine synthesis pathway and angiogenesis in patients with endometriosis for the first time will close the gap in this area to a great extent.

Funding

This work is supported by the Scientific Research Project Fund of Sivas Cumhuriyet University under the project number T-710.

Conflict of interest

All authors declare no conflict of interest.

REFERENCES

1. Gerner EW, Meyskens FL. Polyamines and cancer: old molecules, new understanding. Nat Rev Cancer. 2004; 4(10): 781–792, doi: 10.1038/nrc1454, indexed in Pubmed: 15510159.
2. MOINARD C, CYNOBER L, DEBANDT J. Polyamines: metabolism and implications in human diseases. Clinical Nutrition. 2005; 24(2): 184–197, doi: 10.1016/j.clnu.2004.11.001.
3. Thomas T, Thomas TJ. Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. Cell Mol Life Sci. 2001; 58(2): 244–258, doi: 10.1007/PL00000852, indexed in Pubmed: 11289306.
4. Jeevanandam M, Petersen SR. Clinical role of polyamine analysis: problem and promise. Curr Opin Clin Nutr Metab Care. 2001; 4(5): 385–390, doi: 10.1097/00075197-200109000-00008, indexed in Pubmed: 11568499.
5. Seiler N. Catabolism of polyamines. Amino Acids. 2004; 26(3): 217–233, doi: 10.1007/s00726-004-0070-z, indexed in Pubmed: 15221502.
6. Li Li, Rao J, Guo X, et al. Polyamine depletion stabilizes p53 resulting in inhibition of normal intestinal epithelial cell proliferation. American Journal of Physiology-Cell Physiology. 2001; 281(3): C941–C953, doi: 10.1152/ajpcell.2001.281.3.c941.
7. Liang M, Ekblad E, Hellstrand P, et al. Polyamine synthesis inhibition attenuates vascular smooth muscle cell migration. J Vasc Res. 2004; 41(2): 141–147, doi: 10.1159/000077133, indexed in Pubmed: 15010577.
8. Hoet P, Nemery B. Polyamines in the lung: polyamine uptake and polyamine-linked pathological or toxicological conditions. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2000; 278(3): L417–L433, doi: 10.1152/ajplung.2000.278.3.l417.
9. Ray R, Viar M, Yuan Q, et al. Polyamine depletion delays apoptosis of rat intestinal epithelial cells. American Journal of Physiology-Cell Physiology. 2000; 278(3): C480–C489, doi: 10.1152/ajpcell.2000.278.3.c480.
10. Williams K. Interactions of polyamines with ion channels. Biochem J. 1997; 325 (Pt 2): 289–297, doi: 10.1042/bj3250289, indexed in Pubmed: 9230104.
11. Pfeffer LM, Yang CH, Murti A, et al. Polyamine depletion induces rapid NF-kappa B activation in IEC-6 cells. J Biol Chem. 2001; 276(49): 45909–45913, doi: 10.1074/jbc.M108097200, indexed in Pubmed: 11590175.
12. Shearer JD, Richards JR, Mills CD. Differantial regulation of macrophage arginine metabolism: a proposed role in wound healing. Am J Physiol. 1997; 272(35): E181–E190, doi: 10.1152/ajpendo.1997.272.2.E181, indexed in Pubmed: 9124321.
13. Feng Y, Halaris A, Piletz J. Determination of agmatine in brain and plasma using high-performance liquid chromatography with fluorescence detection. Journal of Chromatography B: Biomedical Sciences and Applications. 1997; 691(2): 277–286, doi: 10.1016/s0378-4347(96)00458-6.
14. Yang Y, Yuan Y, Ma X, et al. Ca2+ channel subunit a 1D inhibits endometriosis cell apoptosis and mediated by prostaglandin E2. Ginekol Pol. 2019; 90(12): 669–674, doi: 10.5603/GP.2019.0115 , indexed in Pubmed: 31909458.
15. Munksgaard PS, Blaakaer J. The association between endometriosis and ovarian cancer: a review of histological, genetic and molecular alterations. Gynecol Oncol. 2012; 124(1): 164–169, doi: 10.1016/j.ygyno.2011.10.001, indexed in Pubmed: 22032835.
16. Gökmen SS, Yörük Y, Cakir E, et al. Arginase and ornithine, as markers in human non-small cell lung carcinoma. Cancer Biochem Biophys. 1999; 17(1-2): 125–131, indexed in Pubmed: 10738908.
17. Leu SY, Wang SR. Clinical significance of arginase in colorectal cancer. Cancer. 1992; 70(4): 733–736, doi: 10.1002/1097-0142(19920815)70:4<733::aid-cncr2820700403>3.0.co;2-6.
18. Harris BE, Pretlow TP, Bradley Jr EL, et al. Arginase activity in prostatic tissue of patients with benign prostatic hyperplasia and prostatic carcinoma . Cancer Res. 1983; 43(6): 3008–3012, indexed in Pubmed: 6189588.

19. Löser C, Fölsch U, Paprotny C, et al. Polyamine Concentrations in Pancreatic Tissue, Serum, and Urine of Patients with Pancreatic Cancer. Pancreas. 1990; 5(2): 119–127, doi: 10.1097/00006676-199003000-00001.
20. Gökmen SS, Aygit AC, Ayhan MS, et al. Significance of arginase and ornithine in malignant tumors of the human skin. J Lab Clin Med. 2001; 137(5): 340–344, doi: 10.1067/mlc.2001.114543, indexed in Pubmed: 11329531.
21. Wu CW, Chi CW, Ho CK, et al. Effect of arginase on splenic killer cell activity in patients with gastric cancer. Dig Dis Sci. 1994; 39(5): 1107–1112, doi: 10.1007/BF02087565, indexed in Pubmed: 8174424.
22. Proenza A, Oliver J, Palou A, et al. Breast and lung cancer are associated with a decrease in blood cell amino acid content. The Journal of Nutritional Biochemistry. 2003; 14(3): 133–138, doi: 10.1016/s0955-2863(02)00225-5.
23. Mohan RR, Challa A, Gupta S, et al. Overexpression of Ornithine Decarboxylase in Prostate Cancer and Prostatic Fluid in Humans, Clinical Cancer Research. Clin Cancer Res. 1999; 5(1): 143–147, indexed in Pubmed: 9918212.
24. Deng W, Jiang X, Mei Yu, et al. Role of ornithine decarboxylase in breast cancer. Acta Biochim Biophys Sin (Shanghai). 2008; 40(3): 235–243, doi: 10.1111/j.1745-7270.2008.00397.x, indexed in Pubmed: 18330478.
25. Yoshida M, Hayashi H, Taira M, et al. Elevated expression of the ornithine decarboxylase gene in human esophageal cancer . Cancer Res. 1992; 52(23): 6671–6675, indexed in Pubmed: 1423312.
26. Satriano J, Matsufuji S, Murakami Y, et al. Agmatine suppresses proliferation by frameshift induction of antizyme and attenuation of cellular polyamine levels. J Biol Chem. 1998; 273(25): 15313–15316, doi: 10.1074/jbc.273.25.15313, indexed in Pubmed: 9624108.
27. Regunathan S, Youngson C, Raasch W, et al. Imidazoline receptors and agmatine in blood vessels: a novel system inhibiting vascular smooth muscle proliferation . Pharmacol Exp Ther. 1996; 276(3): 1272–1282, indexed in Pubmed: 8786560.
28. Xavier P, Belo L, Beires J, et al. Serum levels of VEGF and TNF-alpha and their association with C-reactive protein in patients with endometriosis. Arch Gynecol Obstet. 2006; 273(4): 227–231, doi: 10.1007/s00404-005-0080-4, indexed in Pubmed: 16208475.
29. Vodolazkaia A, Yesilyurt BT, Kyama CM, et al. Vascular endothelial growth factor pathway in endometriosis: genetic variants and plasma biomarkers. Fertil Steril. 2016; 105(4): 988–996, doi: 10.1016/j.fertnstert.2015.12.016, indexed in Pubmed: 26773192.
30. Yu-Hsien Lin K, Yin-Yi Chang C, Lin WC, et al. Increased risk of endometriosis in patients with endometritis- a nationwide cohort study involving 84,150 individuals. Ginekol Pol. 2020; 91(4): 193–200, doi: 10.5603/GP.2020.0040, indexed in Pubmed: 32374019.

VIA MEDICA

ORIGINAL PAPER / GYNECOLOGY

Ginekologia Polska
2023, vol. 94, no. 2, 95–100

ISSN 0017–0011, e-ISSN 2543–6767

DOI 10.5603/GP.a2022.0113

Laparoscopy versus open surgery for the surgical management of tubo-ovarian abscess (TOA). Is there a beneficial impact of early endoscopic intervention in terms of fertility rates?

Meltem Sönmezer[1], Koray Görkem Saçıntı[2], Bulut Varlı[2], Yavuz Emre Şükür[2], Çağrı Gülümser[1], Batuhan Özmen[2], Cem Somer Atabekoğlu[2], Bülent Berker[2], Ruşen Aytaç[2], Murat Sönmezer[2]

[1]Department of Obstetrics and Gynecology, Yuksek Ihtisas University Faculty of Medicine, Turkey
[2]Department of Obstetrics and Gynecology, Faculty of Medicine, Ankara University, Turkey

ABSTRACT

Objectives: To compare success rates and complications in women undergoing laparoscopic versus open surgical management of tubo-ovarian abscess. We further examined whether early laparoscopic intervention has any impact on pregnancy rates in a subgroup of infertile patients following frozen-thawed embryo transfer.

Material and methods: Hospital records of 48 patients diagnosed with TOA between January 2015 and December 2020, who underwent surgical intervention or received only medical treatment were analyzed. All patients were hospitalized, and parenteral antibiotics were commenced on admission initially. Laparoscopic or open surgery was performed within 48 hours course of intravenous antibiotherapy (early intervention) or later according to the clinical findings and antibiotherapy response.

Results: Of 48 patients with TOA, 18 (37.5%) underwent laparoscopic and 30 (62.5%) underwent open surgical intervention. The median postoperative hospital stay was shorter (4.5 days vs 7.5 days, respectively; p = 0.035), and postoperative opioid analgesic requirement was lesser in the laparoscopy group compared to open surgery group (22% vs 53%, respectively; p = 0.034). Intra- and post-operative complication rates were similar between the groups. Of these 48 patients, seven were diagnosed to have TOA following oocyte retrieval, and four of these conceived with frozen thawed embryo transfer all of whom underwent laparoscopic surgery within 48 hours of diagnosis.

Conclusions: Minimal invasive surgery should be preferred even in the presence of severely adhesive and inflammatory TOA in order to improve postoperative outcomes. Moreover, early laparoscopic intervention may be considered in infertile patients with an aim to optimize pregnancy rates in a subsequent frozen-thawed embryo transfer.

Key words: laparoscopy; open surgery; pelvic inflammatory disease; tubo-ovarian abscess

Ginekologia Polska 2023; 94, 2: 95–100

INTRODUCTION

Tubo-ovarian abscess (TOA) is usually a complication of pelvic inflammatory disease. It also occurs as sequalae complicated appendicitis or diverticulitis and following pelvic surgery, and can be severe and life-threatening [1]. Almost one-third of patients hospitalized for pelvic inflammatory disease develop a tubo-ovarian abscess. Although old databases reported a mortality rate between 1.7 and 7.1% for PID, the mortality rated by Centers for Disease Control and Prevention (CDC) from PID was less than 1% in 2017 [2]. TOAs are polymicrobial infections usually caused by a combination of aerobic, anaerobic, and facultative pathogens. Even though first-line management of TOAs is antibiotherapy with success rates approaching 70%, some patients especially with bigger abscess formation do not respond to antibiotherapy well and require some type of surgical intervention [3]. Historical-

Corresponding author:
Murat Sönmezer
Department of Obstetrics and Gynecology, Faculty of Medicine, Ankara University, Turkey
e-mail: msonmezer@gmail.com

Received: 27.06.2022 Accepted: 9.09.2022 Early publication date: 22.11.2022

ly, the gold standard treatment is abdominal hysterectomy together with Salpingo-Oophorectomy [4]. However, many patients with tubo-ovarian abscess occur in reproductive aged women with significant concerns regarding future fertility seeking for fertility sparing surgical options.

Transvaginal ultrasound or computed tomography-guided abdominal drainage and laparoscopic surgery are well established minimally invasive modalities in the management of TOAs. Even though there are certain benefits of image-guided drainage in the short term, such as minimal or no anesthetic risk and less surgical risks [5], long-term impacts especially on fertility outcomes compared to surgical treatment have not been well studied. With advanced technology and greater experience, minimally invasive surgery has been increasingly performed safely and effectively in the management of pelvis abscess.

MATERIAL AND METHODS

In this retrospective cohort study, conducted January 2015 and March 2020, a total of 48 women who underwent surgical treatment for tubo-ovarian abscess (TOA) at the Ankara University Cebeci Hospital and Ankara HRS Women's Hospital were included. The inclusion criterion was TOA diagnosis based on history, physical examination, ultrasonography images, and laboratory findings. The exclusion criteria were non-surgical treatment, including only antibiotherapy and the absence of full follow-up data.

All patients were hospitalized, and parenteral antibiotics were commenced on admission. They initially received an empiric antibiotic protocol, including clindamycin plus gentamicin or cephalosporin and metronidazole. Patients with a history of antibiotic treatment before admission and persistent fever beyond 48 hours were treated by imipenem or piperacillin-tazobactam. A laparoscopic or open surgery was performed within 48 hours course of intravenous antibiotherapy (early intervention) or later according to the clinical findings. The surgical interventions included a combination of abscess drainage, copious saline irrigation, adhesiolysis, salpingectomy, salpingo-oophorectomy or hysterectomy as indicated in surgical exploration findings and according to patient's fertility desire.

Table 1. Demographics of the study and control group

	Laparoscopy (n = 18)	Laparotomy (n = 30)	p
Age, years mean (range)	43 (31–61)	53.5 (32–88)	0.006
Gravidity, n (range)	3 (0–4)	3.5 (1–6)	0.376
Parity, n (range)	2 (0–4)	3 (0–4)	0.131
Contraception, n (%)			0.276
None	4 (22.2)	2 (6.7)	
Menopause	1 (5.6)	5 (16.7)	
Intrauterine device	2 (11.1)	1 (3.3)	
Coitus interruptus	0 (0.0)	1 (3.3)	
Infertility, n (%)	2 (15.4)	0 (0)	0.174
Diabetes Mellitus, n (%)	0 (0)	2 (20)	0.136
Hypertension, n (%)	3 (27)	0 (0)	0.074
Cardiovascular disease, n (%)	0 (0)	1 (100)	0.283
Previous pelvic inflammatory disease, n (%)	5 (27.8)	8 (26.7)	0.933
Previous ovarian surgery, n (%)	0	1	0.283
Appendectomy, n (%)	3	1	0.314
Uterine surgery, n (%)	0	3	0.074
Preoperative antibiotics, n (%)			0.243
Cephalosporin and metronidazole	10 (55.5)	14 (46.6)	
Gentamycin and clindamycin	7 (70)	5 (35.7)	
Other penicillin group drugs and metronidazole	1 (10)	4 (28.6)	
	2 (20)	5 (35.7)	
Preoperative antibiotic treatment, days (range)	4.5 (2–8)	3 (1–24)	0.354
US findings n (%)			0.185
No additional finding	12 (66.6)	19 (86.4)	
Endometrioma	3 (16.6)	1 (4.5)	
Ovarian cancer	0 (0)	2 (9.1)	
Bilaterality of the abscess, n (%)	6 (33.3)	13 (43.3)	0.703
CT performed, n (%)	3 (17.6)	14 (82.4)	0.060
Abscess size, n (%)			0.012
0–4 cm	3 (33.3)	1 (6.7)	
5–8 cm	5 (55.6)	3 (20.0)	
< 8 cm	1 (11.1)	11 (73.3)	

CT — computed tomography; US — ultrasound

RESULTS

Among a total of 48 patients with TOA, 18 patients (37.5%) underwent laparoscopy, and 30 patients (62.5%) underwent open intervention. Main presenting symptoms and findings were pelvic pain (52%), uterine tenderness (14.5%), cervical discharge (0.06%), abnormal bleeding (0.02%), and fever (16.6%). The median age was significantly higher in the open surgery group than the laparoscopy group (53.5 vs 34 years, respectively; p = 0.006). The duration and type of preoperative antibiotic use, preoperative white blood cell count, and serum CRP levels were all similar between the groups. The size of the abscess was also similar between the groups (Tab. 1). The type of surgical intervention was drainage in six patients (33.3%) and nine patients (30%), salpingectomy in 10 patients (55.6%) and seven (23.3%) patients, hysterectomy along with bilateral or unilateral salpingo-oophorectomy in one patient (11.1%) and 13 patients (43.3%) in laparoscopy and open surgery groups respectively (p > 0.05). Moderate to intense adhesiolysis was performed in all patients. There

Table 2. Main operative and postoperative findings

	Laparoscopy (n = 18)	Laparotomy (n = 30)	p
Operative findings			
Type of operation, n (%) Drainage Salpingectomy Hysterectomy + BSO/USO Others	 6 (33.3) 10 (55.6) 2 (11.1) 0 (0)	 9 (30) 7 (23.3) 13 (43.3) 1 (3.3)	0.95
Duration of operation	95 (40–135)	90 (40–150)	0.341
Bowel injury, n (%)	0 (0)	3 (10.0)	0.166
Bladder injury, n (%)	0 (0)	0 (0)	N/A
Postoperative findings			
Follow-up			
Postoperative WBC [10^9/L] (range)	13.5 (7.1–15.3)	12.9 (7.8–20.6)	0.741
Postoperative CRP, mg/L (range)	98 (10.4–313)	106.9 (17.7–313)	0.552
Postoperative Fever, °C (range)	37.4 (36.2–38.1)	36.7 (36.5–37.2)	0.075
Postoperative antibiotics Cephalosporin and metronidazole Gentamycin and clindamycin Piperacillin–tazobactam Other penicillin group drugs and metronidazole	 11(61.1) 0 (0) 1 (5.6) 6 (33.3)	 13 (43.3) 5 (16.6) 1 (3.3) 10 (33.3)	0.217
Duration of postop. antibiotic treatment [days]	10 (7–23)	13 (6–28)	0.150
Hospital stay, days (range)	4.5 (1–16)	7.5 (1–28)	0.035
Analgesia			
Postoperative diclofenac sodium [mg] (range)	262.5 (75–1575)	383.3 (150–1350)	0.715
Postoperative opioid [mg] (range)	0 (0–500)	100 (0–900)	0.013
No. of patients required opioid, n (%)	4 (22.2)	16 (53.3)	0.034
Postoperative complications			
Subileus, n (%)	0 (0)	0 (0)	N/A
Bacteremia, n (%)	0 (0)	0 (0)	N/A
Surgical site infection, n (%)	0	0	N/A
Blood transfusion, n (%)	5 (27.8)	6 (20.0)	0.535

BSO/USO — bilateral salpingo-oophorectomy/ unilateral salpingo--oophorectomy; WBC — white blood cell count; CRP — C-reactive protein; N/A — not applicable

were no significant differences in intraoperative or postoperative complication rates (Tab. 2).

There was no difference in the duration of operation according to surgical approach (95 min. for laparoscopy vs 90 min. for open surgery; p = 0.341) and blood products' transfusion (27.8% vs 20.0%, respectively; p = 0.535) (Tab. 2).

Table 3. Impact of early surgical intervention on operative and post-operative outcome

	Early surgery (n = 22)	Surgery after 48 h (n = 26)	p
Duration of operation	90 (40–130)	90 (40–150)	0.127
Postoperative WBC [10^9/L] (range)	12.8 (7.1–18.5)	15.3 (7.8–20.6)	0.482
Postoperative CRP [mg/L] (range)	91.9 (10.4–173.5)	202.1 (13.1–313)	0.122
Postoperative Fever [°C] (range)	36.5 (36.1–36.9)	37.2 (36.5–38.1)	0.702
Duration of postoperative antibiotic treatment [days]	10 (6–17)	14 (7–28)	< 0.001
Hospital stay [days] (range)	5 (1–10)	7 (1–28)	0.001

WBC — white blood cell count; CRP — C-reactive protein

However, median postoperative hospital stay was significantly shorter in patients who underwent laparoscopy compared to patients managed with open surgery (4.5 days vs 7.5 days, respectively; p = 0.035). Although not statistically significant, there were three intraoperative bowel injuries (10%) in the open surgery group and none in the laparoscopy group. Minor serosal bowel injuries were all repaired by serosal suturing.

There were no complications including bladder injury, ileus, and bacteremia in any of the groups. There was less postoperative analgesic requirement including opioids in the laparoscopy group compared to open surgery group (22% patients in laparoscopy group vs 53% in the open surgery group; p = 0.034). One patient in the laparoscopy group experienced pulmonary embolism. There were no secondary surgeries, perioperative deaths, bacteremia, ileus, bladder/ureteric injury, and vascular injury in both groups.

In a further analysis, we explored the impact of early surgical intervention (within 48 hours of antibiotherapy) on postoperative outcome parameters (Tab. 3). Among all, 22 patients underwent early surgical intervention either laparoscopically or open. The postoperative laboratory parameters, analgesic requirements, and complication rates were similar between the groups. However, both median postoperative hospital stays (5 days vs 7 days; p = 0.001) and duration of postoperative antibiotic course (10 days vs 14 days; p < 0.001) were significantly shorter in the early surgical intervention group (Tab. 3). In the follow up data, we observed that 4/7 patients all of which developed TOA following oocyte retrieval and underwent early laparoscopic intervention within 48 hours of diagnosis and commencement of antibiotic treatment conceived with subsequent frozen-thawed embryo transfer. Moreover, 3 of 4 patients had ovarian endometriomas ranging between 5–7 cm.

DISCUSSION

In the current study, we demonstrated that laparoscopy could be safely and effectively performed in the management of tubo-ovarian abscess. We found that hospital stay is shorter and analgesic requirement is lesser in laparoscopy group compared to open surgery group with similar intraoperative and postoperative complication rates. Moreover, we observed that early surgical intervention has advantageous in terms of length of hospital stay and postoperative antibiotic use, and possibly fertility rates.

The treatment approach for pelvic abscess in reproductive-aged women is highly dependent on clinical presentation, patient characteristics, and desire to preserve future childbearing potential. Despite some published guidelines on PID and tubo-ovarian abscess by various societies, there are no clear-cut recommendations and uniform approach for surgical treatment [6, 7]. Even though most surgeons historically performed laparotomic approach, laparoscopic surgery has been practiced as a safe and effective method. Some guidelines recommend that laparoscopy may provide early resolution of the disease by dividing adhesions and draining abscesses [8]. Following widespread use of endoscopic surgery, multiple advantages have emerged including shorter hospital stay, cosmetic incisions, and lower surgical site infection rates compared to open surgery. Moreover, laparoscopy offers direct visualization providing complete diagnostic accuracy. On the other hand, laparoscopic surgery may be technically challenging in some patients with pelvic abscess due to severe adhesions and obliterated pelvic cavity which requires high surgical skills and experience.

An early intervention strategy including drainage along with intravenous antibiotics seems the best treatment approach to avoid long term complications of TOAs. In a prospective cohort study, CRP was demonstrated to be a sensitive and specific inflammatory marker for predicting TOA in patients with complicated PID, which significantly correlated with success or failure of conservative management [9]. To et al. [5] demonstrated that patients who received antibiotics alone were more likely to require further surgical intervention when compared with patients who additionally received image-guided drainage. In their study treatment selection was not affected by the presence of bilateral presence of abscess. For the long-term follow-up data, there were no differences between the groups in terms of residual pain, pregnancy outcomes, or infertility. The fertility rates were reported as 56.6% in the antibiotic-only group compared to 41.2% in the drainage group. However, it should be noted that abscess size was larger in drainage group compared to antibiotic-only group (8.5 vs 5.9 cm) which may be attributed to the fact that patients in the antibiotic-only arm generally presented with less severe disease. Notably, mean duration of hospital stay was longer in drainage group compared to antibiotic only group (13.3 vs 7.4 days). Size of the abscess, age of the patient, white blood cell count and serum CRP levels are found as important parameters for antibiotic failure in the studies.

Many studies demonstrated that image guided drainage alone or together with intracavitary antibiotic irrigation had high success rates in terms of symptom resolution and no requirement for any additional intervention [10–12]. In a systematic review enrolling a total of 975 patients aged 11 to 86 years, it was found that image-guided drainage of TOAs was associated with highest success rates, fewer complications, and shorter hospital stays compared with laparoscopy [3]. In contrast, other studies found shorter hospital stay with laparoscopy compared to image guided drainage. However, there is a great heterogeneity in terms of success rates, complications, and duration of hospitalization and not consistently reported in the studies. Moreover, the average abscess size ranged from 4.32 to 8.50 cm and the period for improvement on antibiotics alone before the intervention was not standard which varied between 0 and 6 days. In the primary management of tubo-ovarian abscess, we opted to use laparoscopic surgery instead of image guided drainage due to high clinical experience in both laparoscopy and open surgery. Evet though we don't have our own clinical data comparing laparoscopic surgery and image guided drainage, we have quite favorable results with surgical approach.

Doganay et al. reported an average duration of hospital stay as two days for laparoscopy, 7.4 days for laparotomy, and 11 days antibiotics-only treatment [13]. Similarly, Yang et al. [14] also showed a marked decrease in hospital stay (5.4 days vs 8.9 days; $p < .001$) and wound infections with laparoscopy compared to laparotomy. Regarding the complication rates Carlson et al. found that a laparoscopic surgical approach was significantly associated with a lower risk of perioperative complications compared to open surgery [15]. Even though we did not find any significant difference in surgical complication rates, bowel injury only occurred in 3 patients in the open surgery group. Conversion rates to laparotomy are strictly dependent on surgical skill, endoscopic experience, and severity of the adhesions. In a retrospective case series study, which compared the outcomes of 37 patients who were diagnosed with TOA after fertility treatments with 313 women who were diagnosed with TOA not associated with fertility treatments, conversion to laparotomy was more common in patients with endometriosis [16]. In our study three of laparoscopic TOA patients (16.6%) had endometrioma related tubo-ovarian abscess, however there was not any case of conversion to laparotomy. This is most probably due to the fact that all of the operations were performed by highly experienced laparoscopic surgeons.

The morbidity rates associated with surgical management of TOA were reported between 0.8% and 57% [15]. Carlson et al. [15] demonstrated that almost half of the patients treated with initial conservative management underwent laparoscopic exploration. Notably, studies showed that early surgical management has some certain advantages over late surgical intervention in terms of shorter hospital stay, earlier resolution of fever and less blood loss [17]. Similarly, in the current study we observed that when laparoscopy was performed within two days of diagnosis and antibiotherapy, the duration of postoperative hospital stay, and the length of postoperative antibiotic treatment were shorter. A thorough assessment of risk factors and identifying patients who may not respond to initial medical management would allow a prompt surgical intervention that provides a less complicated surgery with lower morbidity rates. In a large study including 4419 patients, the laparoscopic group had shorter operation duration (125 vs 166 min), fewer blood transfusions (4.7% vs 10.0%), and shorter length of hospital stay (5 vs 7 days; $p < 0.001$) compared with the open surgery group (2). As similar, despite similar operation times, the mean duration of hospital stay was shorter in the laparoscopy group compared to open surgery group in our study (4.5 vs 7.5 days; $p = 0.035$).

Tubo-ovarian abscess can also occur following oocyte retrieval, due to direct inoculation of vaginal microorganisms, from an already existing PID, and direct needle puncture of a bowel segment. Although the true incidence of pelvic abscess after oocyte pick up is unknown, it ranges between 0.03% and 0.24% in reported studies [18, 19]. With increasing number of patients with endometriomas undergoing IVF, it has been more common to see endometrioma related pelvic abscess following oocyte pick up. In a retrospective study, it was demonstrated that pelvic inflammatory disease in women with endometriosis is more severe and refractory to antibiotic treatment, and more frequently required surgical intervention [20]. Similarly, in another case series, women with TOA after fertility treatments had severe clinical presentation and complicated clinical course compared with women with TOA not associated with fertility treatments [16]. Furthermore, the rate of surgical intervention, conversion rates to laparotomy and complication rates were significantly higher in patients with endometriosis. However, in a four year follow up study Villete et al. demonstrated that the onset of infectious processes in women with endometriosis is high variable, ranging from 4 to 120 days, and can also occur long after ART or even spontaneously [21]. Despite data are scarce regarding the impact of pelvic inflammation on the outcome of fertility treatments, the reported clinical pregnancy rates are extremely lower when TOA occurs following an ART cycle. In two different studies, pregnancy rates were reported as low as 0 and 9% in patients who develop pelvic abscess, compared to 29 and 30% in the control group without pelvic inflammation [19, 22]. Not only the presence of bacterial toxins may result in direct damage to the embryo, but also the pelvic inflammation can impair embryo implantation via cytokine secretion [23]. However, it is known whether copious irrigation of the pelvic cavity, resecting adhesions, removing all necrotic debris and abscess capsule has any further advantage over image guided drainage in terms of fertility rates. Regarding the long-term fertility rates, Buchweitz et al. found that while 3/16 patients (18.75%) in organ-preserving group achieved live births, only 1/24 women (4.1%) conceived in the non-organ preserving group. Moreover, the rate of complications was significantly higher in the group undergoing ablative treatment compared to organ preserving surgery group [24]. In the current study in a further subgroup analysis, we observed that four patients who underwent early laparoscopic intervention conceived in subsequent frozen-thawed embryo transfer cycles. Of these four patients, three had ovarian endometriomas. As some studies reported more extensive pelvic inflammation and increased incidence of conversion to laparotomy in patients with endometrioma, these specific group of patients may benefit from early surgical intervention in terms of pregnancy outcome.

CONCLUSIONS

We did not find any difference in complication rates, mean operation time, requirement for blood transfusion or surgical site infections between laparoscopy and open surgery groups. However, duration of antibiotic treatment and the length of hospital stay is shorter and analgesic requirement is lesser in laparoscopy group compared to open surgery. We suggest that laparoscopic management of tubo-ovarian abscess has some certain benefits over open surgery in experienced hands. It is still not clear whether early surgical intervention with removal all necrotic material and copious irrigation has benefit in terms of fertility rates over image guided drainage in the long term and should be clarified in further studies. However, it is important such a study requires a long follow up in a specific group of reproductive patients asking for future fertility.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Ethics approval and consent to participate

This current study was approved by the Institutional Review Board (date: 29/12/2020; no: E.2733).

Conflicts of interest

The authors declare that they have no competing interests.

REFERENCES

1. Munro K, Gharaibeh A, Nagabushanam S, et al. Diagnosis and management of tubo-ovarian abscesses. The Obstetrician & Gynaecologist. 2018; 20(1): 11–19, doi: 10.1111/tog.12447.
2. Shigemi D, Matsui H, Fushimi K, et al. Laparoscopic Compared With Open Surgery for Severe Pelvic Inflammatory Disease and Tubo-Ovarian Abscess. Obstet Gynecol. 2019; 133(6): 1224–1230, doi: 10.1097/AOG.0000000000003259, indexed in Pubmed: 31135738.
3. Goje O, Markwei M, Kollikonda S, et al. Outcomes of Minimally Invasive Management of Tubo-ovarian Abscess: A Systematic Review. J Minim Invasive Gynecol. 2021; 28(3): 556–564, doi: 10.1016/j.jmig.2020.09.014, indexed in Pubmed: 32992023.
4. Pedowitz P, Bloomfield R. Ruptured adnexal abscess (tuboovarian) with generalized peritonitis. American Journal of Obstetrics and Gynecology. 1964; 88(6): 721–729, doi: 10.1016/0002-9378(64)90604-0.
5. To J, Aldape D, Frost A, et al. Image-guided drainage versus antibiotic-only treatment of pelvic abscesses: short-term and long-term outcomes. Fertil Steril. 2014; 102(4): 1155–1159, doi: 10.1016/j.fertnstert.2014.06.025, indexed in Pubmed: 25064405.
6. Minakami H, Hiramatsu Y, Koresawa M, et al. Japan Society of Obstetrics and Gynecology, Japan Association of Obstetricians and Gynecologists. Guidelines for obstetrical practice in Japan: Japan Society of Obstetrics and Gynecology (JSOG) and Japan Association of Obstetricians and Gynecologists (JAOG) 2011 edition. J Obstet Gynaecol Res. 2011; 37(9): 1174–1197, doi: 10.1111/j.1447-0756.2011.01653.x, indexed in Pubmed: 21917078.
7. Workowski KA, Bolan GA. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep. 2015; 64(RR-03): 1–137, indexed in Pubmed: 26042815.
8. Ross J, McCarthy GUK. National guideline for the management of pelvic inflammatory disease. British Association for Sexual Health and HIV. ; 2011.
9. Ribak R, Schonman R, Sharvit M, et al. Can the Need for Invasive Intervention in Tubo-ovarian Abscess Be Predicted? The Implication of C-reactive Protein Measurements. J Minim Invasive Gynecol. 2020; 27(2): 541–547, doi: 10.1016/j.jmig.2019.04.027, indexed in Pubmed: 31479751.
10. Caspi B, Zalel Y, Or Y, et al. Sonographically guided aspiration: an alternative therapy for tubo-ovarian abscess. Ultrasound Obstet Gynecol. 1996; 7(6): 439–442, doi: 10.1046/j.1469-0705.1996.07060439.x, indexed in Pubmed: 8807762.
11. Teisala K, Heinonen PK, Punnonen R. Transvaginal ultrasound in the diagnosis and treatment of tubo-ovarian abscess. Br J Obstet Gynaecol. 1990; 97(2): 178–180, doi: 10.1111/j.1471-0528.1990.tb01745.x, indexed in Pubmed: 2180476.
12. Aboulghar MA, Mansour RT, Serour GI. Ultrasonographically guided transvaginal aspiration of tuboovarian abscesses and pyosalpinges: an optional treatment for acute pelvic inflammatory disease. Am J Obstet Gynecol. 1995; 172(5): 1501–1503, doi: 10.1016/0002-9378(95)90486-7, indexed in Pubmed: 7755062.
13. Doganay M, Iskender C, Kilic S, et al. Treatment approaches in tubo-ovarian abscesses according to scoring system. Bratisl Lek Listy. 2011; 112(4): 200–203, indexed in Pubmed: 21585128.
14. Yang IJ, Wu MY, Chao KH, et al. Usage and cost-effectiveness of elective oocyte freezing: a retrospective observational study. Reprod Biol Endocrinol. 2022; 20(1): 123, doi: 10.1186/s12958-022-00996-1, indexed in Pubmed: 35974356.
15. Carlson S, Batra S, Billow M, et al. Perioperative Complications of Laparoscopic versus Open Surgery for Pelvic Inflammatory Disease. J Minim Invasive Gynecol. 2021; 28(5): 1060–1065, doi: 10.1016/j.jmig.2020.08.488, indexed in Pubmed: 32891823.
16. Fouks Y, Cohen Y, Tulandi T, et al. Complicated Clinical Course and Poor Reproductive Outcomes of Women with Tubo-Ovarian Abscess after Fertility Treatments. J Minim Invasive Gynecol. 2019; 26(1): 162–168, doi: 10.1016/j.jmig.2018.06.004, indexed in Pubmed: 29890350.
17. Chu L, Ma H, Liang J, et al. Effectiveness and Adverse Events of Early Laparoscopic Therapy versus Conservative Treatment for Tubo-Ovarian or Pelvic Abscess: A Single-Center Retrospective Cohort Study. Gynecol Obstet Invest. 2019; 84(4): 334–342, doi: 10.1159/000493855, indexed in Pubmed: 30612130.
18. Aragona C, Mohamed MA, Espinola MS, et al. Clinical complications after transvaginal oocyte retrieval in 7,098 IVF cycles. Fertil Steril. 2011; 95(1): 293–294, doi: 10.1016/j.fertnstert.2010.07.1054, indexed in Pubmed: 20727520.
19. Roest J, Mous HV, Zeilmaker GH, et al. The incidence of major clinical complications in a Dutch transport IVF programme. Hum Reprod Update. 1996; 2(4): 345–353, doi: 10.1093/humupd/2.4.345, indexed in Pubmed: 9080231.
20. Elizur SE, Lebovitz O, Weintraub AY, et al. Pelvic inflammatory disease in women with endometriosis is more severe than in those without. Aust N Z J Obstet Gynaecol. 2014; 54(2): 162–165, doi: 10.1111/ajo.12189, indexed in Pubmed: 24576163.
21. Villette C, Bourret A, Santulli P, et al. Risks of tubo-ovarian abscess in cases of endometrioma and assisted reproductive technologies are both under- and overreported. Fertil Steril. 2016; 106(2): 410–415, doi: 10.1016/j.fertnstert.2016.04.014, indexed in Pubmed: 27178227.
22. Yang CC, Chen Pu, Tseng JY, et al. Advantages of open laparoscopic surgery over exploratory laparotomy in patients with tubo-ovarian abscess. J Am Assoc Gynecol Laparosc. 2002; 9(3): 327–332, doi: 10.1016/s1074-3804(05)60412-4, indexed in Pubmed: 12101330.
23. Ashkenazi J, Farhi J, Dicker D, et al. Acute pelvic inflammatory disease after oocyte retrieval: adverse effects on the results of implantation. Fertil Steril. 1994; 61(3): 526–528, doi: 10.1016/s0015-0282(16)56587-x, indexed in Pubmed: 8137978.
24. Buchweitz O, Malik E, Kressin P, et al. Laparoscopic management of tubo-ovarian abscesses. Surgical Endoscopy. 2000; 14(10): 948–950, doi: 10.1007/s004640000249.

VIA MEDICA

ORIGINAL PAPER / GYNECOLOGY

Ginekologia Polska
2023, vol. 94, no. 2, 101–106

ISSN 0017–0011, e-ISSN 2543–6767

DOI 10.5603/GP.a2021.0234

Clinical study of acute toxicity of pelvic bone marrow-sparing intensity-modulated radiotherapy for cervical cancer

Shuangshaung Sun, Zhi Chen, Pingping Li, Jian Wu, Baoling Zhu, Xi Zhang, Congcong Wu, Ruifang Lin, Yingying Zhou, Wenjun Chen

Wenzhou Medical University, China

ABSTRACT

Objectives: To compare the dose volume of the target area and the toxicity of pelvic bone marrow-sparing intensity-modulated radiotherapy (PBMS-IMRT) with routine IMRT in patients undergoing radiochemotherapy for cervical cancer.

Material and methods: Forty patients with indications for adjuvant radiochemotherapy after cervical cancer surgery were selected and randomly divided into IMRT (n = 20) and PBMS-IMRT (n = 20) groups to observe and record the toxicity and its severity in the blood, gastrointestinal tract, and genitourinary system.

Results: There was no significant difference in the target area conformity index (CI) or homogeneity index (HI) between the two groups ($p > 0.05$). The pelvic bone V10–V50 in the PBMS-IMRT group were lower than those in the IMRT group ($p < 0.05$), and there was lower hematological toxicity ($p < 0.05$) and fewer delays or interruptions in chemotherapy and/or radiotherapy ($p < 0.05$) in the PBMS-IMRT group. The toxicity to the gastrointestinal and genitourinary systems in the two groups was not significantly different ($p > 0.05$).

Conclusions: PBMS-IMRT significantly reduced the dose volume of the pelvic bone marrow, thereby reducing the incidence of bone marrow suppression. However, it had no significant impact on the gastrointestinal or genitourinary systems.

Key words: cervical cancer; confined pelvic bone marrow; intensity-modulated radiotherapy; toxicity

Ginekologia Polska 2023; 94, 2: 101–106

INTRODUCTION

Cervical cancer is the third most common malignant tumor in women worldwide, and it has always ranked first among gynecological malignancies in China. In recent years, cisplatin-based concurrent radiochemotherapy has become the standard treatment for advanced cervical cancer. Compared with radiotherapy alone, concurrent radiochemotherapy can reduce mortality by 30–50% [1–3]; however, it is associated with increased toxicity, especially acute hematological toxicity that may cause Grade 3 or higher bone marrow suppression and force patients to stop radiochemotherapy [4, 5]. Approximately 50% of adult bone marrow hematopoiesis is concentrated in the pelvic bone marrow and lower vertebral body [6, 7]. Several studies have shown that effectively reducing the volume of bone marrow irradiated during radiotherapy can reduce the risk of bone marrow suppression in patients with concurrent cervical cancer chemoradiotherapy [6–10]. Based on the above studies, as well as on the need to ensure precise coverage of the tumor target area while protecting organs at risk (OAR), this study clinically observed whether pelvic bone marrow-sparing intensity-modulated radiotherapy (PBMS-IMRT) can reduce acute side effects and ensure the smooth progress of concurrent radiochemotherapy in patients with cervical cancer.

MATERIAL AND METHODS

General clinical information

Forty patients who were admitted to our hospital for the first time after surgery for early cervical cancer between May 2016 and May 2017 were selected and divided into PBMS-IMRT (n = 20) and IMRT (n = 20) groups using the random table method. All selected patients had undergone extensive hysterectomy + pelvic lymph node dissection, were pathologically diagnosed with cervical cancer (squamous cell carcinoma or non-squamous cell carcinoma), and had no preoperative radiotherapy or chemotherapy. The indications for postoperative radiotherapy included:

Corresponding author:
Wenjun Chen
The Second Affiliated Hospital of Wenzhou Medical University, China
e-mail: wenjunchendoc@126.com

Received: 15.01.2021 Accepted: 27.04.2021 Early publication date: 8.02.2022

(1) having one high-risk factor after surgery (positive lymph node metastasis, positive surgical margin, or parauterine infiltration) and (2) having two medium-risk factors after surgery (tumor diameter ≥ 4 cm, interstitial infiltration depth greater than one-third, lymphatic vascular interstitial infiltration, or adenocarcinoma). The postoperative stage was determined jointly by an associate chief physician or above from the Department of Oncology, the Department of Oncology, and the Department of Gynecologic Oncology. The exclusion criteria were as follows: (1) history of hypertension, diabetes, heart disease, liver disease, kidney disease, neurologic diseases, other serious diseases, other tumors, or had received radiotherapy or chemotherapy; (2) no routine blood tests or computed tomography (CT) examination before chemoradiotherapy, abnormal blood indicators before chemoradiotherapy, or distant metastasis on CT; or (3) contraindications to radiotherapy and chemotherapy. The study was conducted in accordance with the principles of the Declaration of Helsinki. This study was approved by the ethics committee of Wenzhou Medical University. Written informed consent was obtained from all participants.

Simulative positioning and target area outline

Each patient was first placed in a fixed position using a vacuum air cushion and then enhanced CT scan positioning was performed using a Volume Zoom CT scanner (Smatom Series, Siemens Healthineers, Erlangen, Germany) with a scanning layer thickness of 5 mm and a scanning range of L3–5 cm under the pubic symphysis. The images were then transmitted to the therapy planning system (TPS) (Pinnacle 9.10 Radiation Therapy Planning System, Philips Healthcare, Amsterdam, Netherlands) to outline the target area. Referring to the Delineation Guidelines issued by the Tumor Radiation Therapy Cooperative Organization (RTOG) [11], the clinical target volume (CTV) of the target area was delineated in the TPS system, including that of the common iliac lymph nodes, internal and external iliac lymph nodes, anterior sacral lymph nodes, obturator lymph nodes, lymphatic cysts (if any), surgical stump, and 3 cm of the proximal vagina; the area 5 mm exterior to the CTV (2 mm to the rectal side) was defined as the planning target volume (PTV). The CTVs of the small intestines, bladder, rectum, spinal cord, and bilateral femoral heads were also delineated. For the PBMS-IMRT group, the CTV of the pelvic bone (all hip bones, sacrococcyx, and upper femurs in the radiation field) was delineated, and the area 5 mm exterior to it was defined as the PTV. The clinical goals of the dose-volume of the OAR were V30 < 38% for the small intestine, V40 < 45% for the bladder, and V50 < 20% for the rectum. The clinical goals of the dose-volume of the OAR in the PBMS-IMRT group were V20 < 76% and V40 < 35% [11]. All 40 patients were treated as planned.

Radiotherapy plan

The treatment plans in the two groups were designed in the TPS system, the 7-field irradiation method was used with an X-ray energy of 6 MV, and a Varian 23EX medical linear accelerator (Varian Medical Systems, Palo Alto, CA) was used to implement the radiotherapy plan. The prescribed dose of the PTV was 50 Gy over 25 fractions for five weeks with a 95% isodose curve surrounding the PTV. Acceptable evaluation plans were assessed by clinicians and physicists based on clinical requirements.

Dosimetric evaluation

Combined evaluation was performed by clinicians and physicists using dose-volume histograms. The homogeneity index (HI) and conformity index (CI) were as follows: HI = D5/D95, CI = V95/PTV, where D5 represents the PTV dose of 5% of the target area, D95 represents the PTV dose of 95% of the target area, and V95 represents the exposure volume enclosed by a 95% isodose surface of the prescribed dose. The HI indicates the dose distribution in the target area; the smaller the value, the more uniform the dose distribution in the target area. The CI indicates the consistency between the area surrounded by the isodose surface and the target area, in a range from 0 to 1; the larger the value, the better the fit.

Chemotherapy

Both the PBMS-IMRT and IMRT groups received concurrent chemotherapy during radiotherapy. The chemotherapy regimen consisted of weekly administration of cisplatin (CDDP) at 35–40 mg/m^2 and radiotherapy on days 1, 8, 15, 22, 29, and 36.

Classification criteria for acute radiation injury

Acute radiation reactions occurred during treatment or within three months after the completion of radiotherapy. The American Radiotherapy Collaborative Group acute radiation injury classification standard [4] was adopted.

Follow-up

The follow-up period was August 2017. Each patient was followed up for at least three months, and the follow-up rate was 100%. For each patient, blood tests were regularly performed in the clinic, and the patients were asked to self-report by telephone about radiation reactions in the digestive and genitourinary systems.

Statistical analysis

The statistical software package SPSS 20.0 (IBM, Armonk, NY) was used to analyze the data, and t-tests were used to compare the target area and the dose-volume parameters in OAR between the two groups. The corrected fourfold table χ^2 test was used to compare the delay and/or interruption rates of

chemotherapy and/or radiotherapy between the two groups. The non-parametric Mann-Whitney U test was used to compare acute reactions in the blood, digestive, and urinary systems. Statistical significance was established at $\alpha = 0.05$ and $p < 0.05$.

RESULTS

The patients were aged between 30–62 years, with a median age of 50 years and Karnofsky Performance Status (KPS) [12] scores of ≥ 90 points, including 12 cases of stage IB1, 9 cases of stage IB2, 8 cases of stage IIA1, and 11 cases of stage IIA2.

Evaluation of the PTV target area coverage

There was no significant difference in CI or HI values between the two groups ($p > 0.05$) (Tab. 1).

Comparison of the dose-volume parameters in OAR

There was a significant difference in dose-volume parameters of the pelvis at various levels between the two groups, but not in the dose-volume parameters of the small intestine, bladder, or rectum between the two groups (Tab. 2).

Table 1. Comparison of CI and HI between groups ($\bar{x} \pm s$)

Group	n	CI	HI
PBMS-IMRT	20	0.863 ± 0.025	0.103 ± 0.024
IMRT	20	0.852 ± 0.030	0.093 ± 0.015
t		1.260	1.580
p		0.215	0.122

CI — conformity index; HI — homogeneity index; PBMS-IMRT — pelvic bone marrow-sparing intensity-modulated radiotherapy; IMRT — intensity-modulated radiotherapy

Comparison of toxicity

The severity of hematological toxicity in the IMRT group was significantly higher than that in the PBMS-IMRT group ($Z = -2.186$, $p = 0.038$). There was no significant difference in the severity of toxicity in the lower digestive tract ($Z = -1.492$, $p = 0.136$) or the urinary tract ($Z = -1.399$, $p = 0.162$) (Tab. 3–5).

Impact on the completion of the chemotherapy and radiotherapy plans

The PBMS-IMRT group had a significantly better delay rate and/or discontinuation rate of chemotherapy and/or radiotherapy than the IMRT group ($p < 0.05$) (Tab. 6).

DISCUSSION

Concurrent radiochemotherapy is mainly used in patients with stage IIB-IVA cervical cancer. Multiple randomized controlled studies have shown that concurrent radiochemotherapy can reduce the risk of death by 30–50% compared to radiotherapy alone. However, concurrent radiochemotherapy can also cause severe bone marrow suppression, thus delaying the completion of treatment plans for cervical cancer, or even causing the suspension of treatment plans in cases with severe bone marrow suppression. This negatively affects the prognosis. Therefore, to reduce toxicity in the blood and ensure completion of the planned

Table 2. Comparison of dose-volume parameters in the endangered organs ($\bar{x} \pm s$)

Group	Dose-volume	PBMS-IMRT	IMRT	t	p
Pelvis	V_{10}	85.98 ± 3.01	90.07 ± 2.83	–4.427	< 0.001
	V_{20}	72.43 ± 4.98	80.02 ± 4.88	–4.868	< 0.001
	V_{30}	52.91 ± 4.34	58.72 ± 5.24	–3.819	< 0.001
	V_{40}	33.63 ± 4.23	38.12 ± 5.97	–2.744	0.005
	V_{50}	11.46 ± 1.33	16.21 ± 3.22	–6.097	< 0.001
Small intestine	V10	80.66 ± 5.30	82.67 ± 5.12	–1.220	0.231
	V20	60.37 ± 7.29	64.59 ± 8.00	–1.744	0.089
	V30	30.28 ± 6.03	30.96 ± 5.27	–0.380	0.706
	V40	11.34 ± 5.62	14.00 ± 5.94	–1.438	0.159
	V50	4.30 ± 1.18	4.27 ± 1.36	0.075	0.941
Bladder	V20	100 ± 0	100 ± 0	0	1.000
	V30	76.43 ± 8.32	75.99 ± 8.65	0.164	0.871
	V40	42.33 ± 7.32	42.20 ± 6.70	0.059	0.954
	V50	15.29 ± 2.02	14.72 ± 1.95	0.908	0.370
Rectum	V20	100 ± 0	100 ± 0	0	1.000
	V30	94.37 ± 2.49	94.11 ± 2.63	0.321	0.321
	V40	46.30 ± 10.34	45.29 ± 8.62	0.340	0.739
	V50	8.92 ± 2.46	8.99 ± 2.50	–0.089	0.929

PBMS-IMRT — pelvic bone marrow-sparing intensity-modulated radiotherapy; IMRT — intensity-modulated radiotherapy

Table 3. Comparison of toxicity in the blood system between groups

Group	n	Hematological toxicity			
		Level 0	Level 1	Level 2	Level 3
PBMS-IMRT	20	4	11	3	2
IMRT	20	2	9	5	4
Z	–2.186				
p	0.038				

PBMS-IMRT — pelvic bone marrow-sparing intensity-modulated radiotherapy; IMRT — intensity-modulated radiotherapy

Table 4. Comparison of toxicity in the lower digestive tract between groups

Group	n	Toxicity in lower digestive tract			
		Level 0	Level 1	Level 2	Level 3
PBMS-IMRT	20	3	12	3	2
IMRT	20	1	10	5	4
Z	–1.492				
p	0.136				

PBMS-IMRT — pelvic bone marrow-sparing intensity-modulated radiotherapy; IMRT — intensity-modulated radiotherapy

Table 5. Comparison of toxicity in the Urinary system between groups

Group	n	Toxicity in urinary system			
		Level 0	Level 1	Level 2	Level 3
PBMS-IMRT	20	4	11	3	2
IMRT	20	2	9	5	4
Z	–1.399				
i	0.162				

PBMS-IMRT — pelvic bone marrow-sparing intensity-modulated radiotherapy; IMRT — intensity-modulated radiotherapy

Table 6. Comparison of delay or discontinuation rates of chemotherapy and/or radiotherapy between groups

Group	Cases with delayed or discontinued chemotherapy and/or radiotherapy	Cases without delay or discontinuation rates of chemotherapy and/or radiotherapy	Sum	Delay or discontinuation rate
PBMS-IMRT	1	19	20	5%
IMRT	8	12	20	40%
X^2	5.161			
p	0.023			

PBMS-IMRT — pelvic bone marrow-sparing intensity-modulated radiotherapy; IMRT — intensity-modulated radiotherapy

concurrent radiochemotherapy, it is necessary to study how to reduce the irradiation volume of the hematopoietic bone marrow [4, 13–16].

More than 50% of the hematopoietic activity in the bone marrow is located in the lumbosacral spine, ilium, ischium, pubis, and proximal femurs, and these areas are exposed to varying degrees during pelvic radiotherapy for cervical cancer. Most studies have confirmed that myelosuppression in patients undergoing pelvic radiochemotherapy is related to the volume of the bone marrow receiving 10 or 20 Gy doses [6, 7]. Zhu et al. [17] analyzed 102 cervical cancer patients receiving pelvic radiotherapy combined with cisplatin chemotherapy (40 mg/m^2/week) in three American centers, none of whom received granulocyte monocyte colony-stimulating factor (GM-CSF) or platelet transfusion therapy. Through the functional logarithmic transformation of time (weeks), they found that the weekly peripheral blood cell counts (*ln* [white blood cells (WBCs)] and *ln* [absolute neutrophil counts (ANC)]) were reduced and that there was a significant correlation between the increase in average photobiomodulation (PBM) radiation doses (V20, V30, and V40) and the weekly reduction of WBC and ANCs. With

each 1-Gy increase in PBM, *ln* (ANC) decreased by 9.6/μL/week (95% confidence interval, 1.9-17.3, p = 0.015). Subgroup analysis revealed a significant association between weekly decreases in *ln*(WBC) and *ln* (ANC) among the lumbosacral spine, ischium, and proximal femur. Therefore, the incidence of acute blood toxicity can be decreased by reducing the dose of pelvic radiation. Three-dimensional chemoradiotherapy (3D-CRT) and IMRT are the two technologies currently used to treat pelvic cancer. IMRT reduces the radiation dose to normal pelvic tissues. Compared with 3D-CRT, although the volume of bone marrow exposed to IMRT is lower, the incidence of bone marrow suppression in patients with cervical cancer undergoing concurrent IMRT and radiochemotherapy is still high. To date, there is no consensus on the ability of IMRT to reduce blood toxicity compared with 3D-CRT technology [18]. At present, most pelvic IMRT radiotherapy plans do not limit pelvis-endangering doses in the radiation field, and physicists have not paid enough attention to further reduce the radiation dose-volume of the pelvis. Lujan et al. [19] proposed a dosimetric study of bone-limited pelvic IMRT (BMS-IMRT) and concluded that it could reduce bone marrow toxicity. Mell et al. [20] conducted a phase II study on bone marrow-sparing RT and Huang et al. [21] reported that PBMS-IMRT reduced the incidence of hematologic toxicity in patients with cervical cancer receiving concurrent chemoradiotherapy. A single-center prospective randomized controlled trial from 2020 is also underway.

In this study, 40 patients with early-stage cervical cancer were divided into IMRT and PBMS-IMRT groups. The comparison of dosimetry and toxicity in the blood, digestive, and urinary systems between the two groups revealed that the severity of hematological toxicity in the IMRT group was significantly higher than that in the PBMS-IMRT group, and the on-time completion of the radiotherapy plan in the IMRT group was significantly worse than that in the PBMS-IMRT group ($p < 0.05$). There were no significant differences in the severity of digestive and urinary system toxicities ($p > 0.05$). The hematological toxicity results of this study are similar to those of Lujan et al. [22] and Gandhi et al. [23], but the results of Mundt et al. [24] could be useful in indicating the toxicity reported in the other studies as numbers. However, this study had a short study period and a small sample size, and signs of radiotoxicity in the lower digestive tract and urogenital system can take months to years to appear [4]. The impact on subacute and chronic toxic reactions, as well as on long-term survival, requires further study.

In summary, PBMS-IMRT significantly reduced radiation exposure to the pelvic bone marrow and reduced the incidence of bone marrow suppression, thus ensuring on-time completion of radiochemotherapy plans. This not only expands the body of research but is also clinically significant. The impact on subacute and chronic toxic reactions as well as on long-term survival requires further study.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

1. Rose PG, Bundy BN, Watkins EB, et al. Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer. N Engl J Med. 1999; 340(15): 1144–1153, doi: 10.1056/NEJM199904153401502, indexed in Pubmed: 10202165.
2. Datta NR, Stutz E, Liu M, et al. Concurrent chemoradiotherapy vs. radiotherapy alone in locally advanced cervix cancer: A systematic review and meta-analysis. Gynecol Oncol. 2017; 145(2): 374–385, doi: 10.1016/j.ygyno.2017.01.033, indexed in Pubmed: 28188016.
3. Vordermark D. Radiotherapy of Cervical Cancer. Oncol Res Treat. 2016; 39(9): 516–520, doi: 10.1159/000448902, indexed in Pubmed: 27614991.
4. Kirwan JM, Symonds P, Green JA, et al. A systematic review of acute and late toxicity of concomitant chemoradiation for cervical cancer. Radiother Oncol. 2003; 68(3): 217–226, doi: 10.1016/s0167-8140(03)00197-x, indexed in Pubmed: 13129628.
5. Nicholas S, Chen L, Choflet A, et al. Pelvic Radiation and Normal Tissue Toxicity. Semin Radiat Oncol. 2017; 27(4): 358–369, doi: 10.1016/j.semradonc.2017.04.010, indexed in Pubmed: 28865519.
6. Mauch P, Constine L, Greenberger J, et al. Hematopoietic stem cell compartment: acute and late effects of radiation therapy and chemotherapy. Int J Radiat Oncol Biol Phys. 1995; 31(5): 1319–1339, doi: 10.1016/0360-3016(94)00430-S, indexed in Pubmed: 7713791.
7. Hui B, Zhang Y, Shi F, et al. Association between bone marrow dosimetric parameters and acute hematologic toxicity in cervical cancer patients undergoing concurrent chemoradiotherapy: comparison of three-dimensional conformal radiotherapy and intensity-modulated radiation therapy. Int J Gynecol Cancer. 2014; 24(9): 1648–1652, doi: 10.1097/IGC.0000000000000292, indexed in Pubmed: 25275663.
8. Bao Z, Wang D, Chen S, et al. Optimal dose limitation strategy for bone marrow sparing in intensity-modulated radiotherapy of cervical cancer. Radiat Oncol. 2019; 14(1): 118, doi: 10.1186/s13014-019-1324-y, indexed in Pubmed: 31378200.
9. Mell LK, Sirák I, Wei L, et al. INTERTECC Study Group. Bone marrow-sparing intensity modulated radiation therapy with concurrent cisplatin for stage IB-IVA cervical cancer: an international multicenter phase II clinical trial (INTERTECC-2). Int J Radiat Oncol Biol Phys. 2017; 97(3): 536–545, doi: 10.1016/j.ijrobp.2016.11.027, indexed in Pubmed: 28126303.
10. Albuquerque K, Giangreco D, Morrison C, et al. Radiation-related predictors of hematologic toxicity after concurrent chemoradiation for cervical cancer and implications for bone marrow-sparing pelvic IMRT. Int J Radiat Oncol Biol Phys. 2011; 79(4): 1043–1047, doi: 10.1016/j.ijrobp.2009.12.025, indexed in Pubmed: 20471182.
11. Lim K, Small W, Portelance L, et al. Gyn IMRT Consortium. Consensus guidelines for delineation of clinical target volume for intensity-modulated pelvic radiotherapy for the definitive treatment of cervix cancer. Int J Radiat Oncol Biol Phys. 2011; 79(2): 348–355, doi: 10.1016/j.ijrobp.2009.10.075, indexed in Pubmed: 20472347.
12. Terret C, Albrand G, Moncenix G, et al. Karnofsky Performance Scale (KPS) or Physical Performance Test (PPT)? That is the question. Crit Rev Oncol Hematol. 2011; 77(2): 142–147, doi: 10.1016/j.critrevonc.2010.01.015, indexed in Pubmed: 20185330.
13. Green JA, Kirwan JM, Tierney JF, et al. Survival and recurrence after concomitant chemotherapy and radiotherapy for cancer of the uterine cervix: a systematic review and meta-analysis. Lancet. 2001; 358(9284): 781–786, doi: 10.1016/S0140-6736(01)05965-7, indexed in Pubmed: 11564482.
14. Chen SW, Liang JA, Hung YC, et al. Concurrent weekly cisplatin plus external beam radiotherapy and high-dose rate brachytherapy for advanced cervical cancer: a control cohort comparison with radiation alone on treatment outcome and complications. Int J Radiat Oncol Biol Phys. 2006; 66(5): 1370–1377, doi: 10.1016/j.ijrobp.2006.07.004, indexed in Pubmed: 16979836.
15. Phillips P, Phillips J. Hysterectomy with radiotherapy or chemotherapy or both for women with locally advanced Cervical Cancer. Clin Nurse Spec. 2017; 31(4): 189–190, doi: 10.1097/NUR.0000000000000304, indexed in Pubmed: 28594668.

16. Kong W, Wang Y, Lv N, et al. Incidence of radiation enteritis in cervical cancer patients treated with definitive radiotherapy versus adjuvant radiotherapy. Journal of Cancer Research and Therapeutics. 2018; 14(8): 120, doi: 10.4103/0973-1482.163762.
17. Zhu He, Zakeri K, Vaida F, et al. Longitudinal study of acute haematologic toxicity in cervical cancer patients treated with chemoradiotherapy. J Med Imaging Radiat Oncol. 2015; 59(3): 386–93; quiz 394, doi: 10.1111/1754-9485.12297, indexed in Pubmed: 25828068.
18. Peng W, Zhao Y. Comparative of patients with cervical cancer using 3D-CRT and IMRT. 2016 9th International Congress on Image and Signal Processing, BioMedical Engineering and Informatics (CISP-BMEI). 2016, doi: 10.1109/cisp-bmei.2016.7853018.
19. Lujan AE, Mundt AJ, Yamada SD, et al. Intensity-modulated radiotherapy as a means of reducing dose to bone marrow in gynecologic patients receiving whole pelvic radiotherapy. Int J Radiat Oncol Biol Phys. 2003; 57(2): 516–521, doi: 10.1016/s0360-3016(03)00521-2, indexed in Pubmed: 12957265.
20. Mell LK, Sirák I, Wei L, et al. INTERTECC Study Group. Bone Marrow-sparing Intensity Modulated Radiation Therapy With Concurrent Cisplatin For Stage IB-IVA Cervical Cancer: An International Multicenter Phase II Clinical Trial (INTERTECC-2). Int J Radiat Oncol Biol Phys. 2017; 97(3): 536–545, doi: 10.1016/j.ijrobp.2016.11.027, indexed in Pubmed: 28126303.
21. Huang J, Gu F, Ji T, et al. Pelvic bone marrow sparing intensity modulated radiotherapy reduces the incidence of the hematologic toxicity of patients with cervical cancer receiving concurrent chemoradiotherapy: a single-center prospective randomized controlled trial. Radiat Oncol. 2020; 15(1): 180, doi: 10.1186/s13014-020-01606-3, indexed in Pubmed: 32727497.
22. Lujan AE, Mundt AJ, Yamada SD, et al. Intensity-modulated radiotherapy as a means of reducing dose to bone marrow in gynecologic patients receiving whole pelvic radiotherapy. Int J Radiat Oncol Biol Phys. 2003; 57(2): 516–521, doi: 10.1016/s0360-3016(03)00521-2, indexed in Pubmed: 12957265.
23. Gandhi AK, Sharma DN, Rath GK, et al. Early clinical outcomes and toxicity of intensity modulated versus conventional pelvic radiation therapy for locally advanced cervix carcinoma: a prospective randomized study. Int J Radiat Oncol Biol Phys. 2013; 87(3): 542–548, doi: 10.1016/j.ijrobp.2013.06.2059, indexed in Pubmed: 24074927.
24. Mundt AJ, Roeske JC, Lujan AE, et al. Initial clinical experience with intensity-modulated whole-pelvis radiation therapy in women with gynecologic malignancies. Gynecol Oncol. 2001; 82(3): 456–463, doi: 10.1006/gyno.2001.6250, indexed in Pubmed: 11520140.

VIA MEDICA

ORIGINAL PAPER / OBSTETRICS

Ginekologia Polska
2023, vol. 94, no. 2, 107–112

ISSN 0017–0011, e-ISSN 2543–6767

DOI 10.5603/GP.a2021.0078

The influence of preincubation time of prepared sperm before IVF on fertilization, embryo developmental competence and the reproductive outcomes

Sai Liu[1, 2], Guoxuan Wu[3], Li Wang[2], Yanyan Zhao[1], Yongqing Lv[2], Nannan Dang[2], Yuanqing Yao[1]

[1]Department of Obstetrics and Gynecology, The First Medical Center of PLA General Hospital, Medical School of Chinese PLA, Beijing, China

[2]Department of Reproductive and Genetics Center, The First Hospital of Kunming Calmette Hospital, Kunming, China

[3]Department of Reproductive Medicine Center, The 988 Hospital of the Joint Logistics Support Force of the Chinese People's Liberation Army, Zhengzhou, China

ABSTRACT

Objectives: It has been provided that if incubation time of prepared sperm can affect sperm motility and DNA fragment, but little is known about the influence of sperm preincubation time (SI) on the sperm's fertilizing ability, subsequent embryonic development and pregnancy outcomes in in vitro fertilization (IVF). The aim of this study was to explore the association of SI with fertilization rate, embryo development and clinical outcomes in IVF, further, to find an optimal preincubation time for prepared sperm before insemination in IVF.

Material and methods: This retrospective cohort study included a total of 1453 infertile couples undergoing IVF in our center performed from January 2016 to January 2019. Sperm were preincubated at 37°C 6% CO_2 for different times before insemination. Preincubation time associated with fertilization rate (FR), 2PN rate, D3 good quality embryo rate, fresh embryo implantation rate (IR), blastocyst formation rate, cumulative pregnancy rate (CPR), cumulative ongoing pregnancy rate (COPR), cumulative live birth rate (CLBR), newborn health and gender ratio were analyzed by chi-square analysis.

Results: FR and 2PN rate of SI more than four hours SI groups (> 4 h SI group) decreased significantly compared with other SI groups ($p < 0.01$). There were no significant differences of the D3 high quality embryo rate among five SI groups. The blastocyst formation rate of > 4 h SI group was significantly lower than that of 2–3 h SI group (45.5% vs 56.1%, $p < 0.05$); and 1–2 h SI group also had significant difference with 2–3 h and 3–4 h SI group (48.9% vs 56.1% and 54.6%, $p < 0.05$). There were a significant decrease of fresh IR and CPR in ≤1 h SI group compared with 1–2 h SI group (19.6% vs 38.0%, $p < 0.05$; 62.7% vs 73.7%, $p < 0.05$); ≤ 1 h SI group also have the lowest CLBR (45.6%), it had statistic differences with 1–2 SI group and 3–4 SI group (45.6% vs 63.2%, $p < 0.01$; 45.6% vs 61.2%, $p < 0.05$).

Conclusions: The sperm preincubated time at 37°C 6% CO_2 before insemination could influence sperm fertilizing ability, blastocyst formation, embryo implantation and CLBR in IVF cycles. The best time for prepared sperm preincubation at 37°C is one to four hours before insemination in IVF.

Key words: sperm; preincubation time; IVF; fertilization rate; embryo development; cumulative live birth

Ginekologia Polska 2023; 94, 2: 107–112

INTRODUCTION

Immediately following ejaculation, human sperm lack the ability to fertilize. To achieve fertilization-competence, sperm must undergo several metabolic and structural changes, collectively known as capacitation [1]. During capacitation, modification of membrane characteristics, enzyme activity, and motility property of spermatozoa render these cells responsive to stimuli that induce the acrosome reaction prior to fertilization [2]. Sperm capacitation is a temperature dependent phenomenon since variations in the incubation temperature cause alterations in key events associated with these processes. At room temperature, sperm cells are in a 'quiescent' state (non-capacitated, with low percentages of spontaneous

Corresponding author:
Yuanqing Yao
Department of Obstetrics and Gynecology, The First Medical Center of PLA General Hospital, Medical School of Chinese PLA, Beijing, China
e-mail: yqyao_ghpla@163.com

Received: 26.11.2020 Accepted: 11.03.2021 Early publication date: 14.05.2021

AR), but when exposure to 37°C, capacitation-related events are activated [3].

Sperm quality is an important predictor of fertility and successful IVF outcomes. To date many studies have been conducted to evaluate the relationship between sperm parameters and sperm incubation time at 37°C. Several studies [4–6] have shown that there is a significant decrease in sperm motility when processed sperm are incubated > four hours before use in IVF. In other studies, a preincubation time of > 6 hours can have deleterious effects on sperm function whereby oxidative stress and reactive oxygen species production increases over a 24-hour period, promoting DNA fragmentation [7]. Further, two groups showed that sperm DNA fragmentation significantly increased, either after a two hour incubation period [8] or after a four-hour incubation period [9]. In other studies, when human sperm were incubated under capacitating conditions up to 24 h, a significant increase in acrosome loss over time was observed [10]. Further, in related experiments, after sperm were preincubated for three hours, it was observed that the percentage of acrosome reactions significantly increased but the spermatozoa ATP concentration did not change between one and three hours [11].

In routine IVF, processed sperm are incubated at 37°C incubator to promote capacitation. Currently, different laboratories select a range of incubation times from as short as under one hour to as long as five hours. To date, there have been no systemic studies to correlate the optimal time for sperm incubation and IVF outcomes. To address this issue, we retrospectively analyzed the relationship between sperm incubation time at 37°C with fertilization, embryonic development and cumulative clinical outcomes in a large cohort of patients.

MATERIAL AND METHODS

IVF patients

This study enrolled 1453 women who had completed 1622 IVF cycles performed from January 2016 and January 2019 at the Department of Reproductive Medicine in Calmette Hospital, Kunming, China. We included hyperstimulation cycles in which the male partner had normal sperm parameters or only mild male factor. Couples were excluded from the study if they had complete fertilization failure, or their fertilization rate was less than 30%. IVF cycles were divided into five test groups according to the prepared sperm incubation time before insemination, namely, ≤ 1h, 1–2h, 2–3h, 3–4h and > 4h.

Ovarian stimulation and laboratory procedures

Women were treated with 1.5 mg of Diphereline (triptorelin acetate for injection; IPSEN PHARMA BIOTECH, France) on day 21 of their previous menstrual cycle. After the serum oestradiol concentration had decreased to < 50 pg/mL, 75–225 IU of recombinant human follitropin (GONAL-f: Merck Serono SA Aubonne Branch, Switzerland) was administered. Oocyte maturation was induced by injection of 5000–10,000 IU of chorionic gonadotrophin (Livzon Pharm, China) when more than three follicles of ≥ 18mm diameter had developed in both ovaries. Cumulus oocyte complexes (COCs) were then retrieved 36h post trigger by ultrasound-guided transvaginal follicular aspiration. COCs were finally collected in buffered medium (G-MOPS® PLUS, Vitrolife, Göteborg) containing Human Serum Albumin (HSA) and then incubated at 37°C in 6% CO_2 and 95% relative humidity (RH) in culture medium containing HSA (IVF®, Vitrolife Sweden AB, Goteborg, Sweden).

On the day of oocyte pick up (OPU), sperm samples were collected from male partners by masturbation following two to seven days of sexual abstinence. Samples were analyzed for sperm count, motility and morphology according to the 2010 parameters of the World Health Organization [12]. After sperm liquefication sperm was purified through a density gradient comprising two 1 mL layers of 40% and 48% Sperm Isolate (Isolate, Ovrine). After deposition of 1–1.5 mL liquefied sperm onto the 40% layer, and centrifugation at 300xg for 10 minutes, the pellet was collected and washed with 2 mL of IVF Plus Medium at 200 g for two minutes. The final pellet was then resuspended in IVF Plus Medium (0.3–1.0 mL) at a final concentration of 3–5 × 10^6 sperm/mL. The whole process was performed at room temperature. Processed sperm was then preincubated at 36°C in 6% CO_2 and 95% relative humidity (RH) for either ≤ 1h, 1–2h, 2–3h, 3–4h or > 4h.

Approximately 10,000 motile sperm were added into 50 µL culture medium drops (IVF®,Vitrolife, Göteborg) four hours after COCs collection (each drop contained one or two COCs). Cumulus cells were subsequently removed at 16–19h post-insemination and the zygotes were washed and transferred to independent drops of 25 µL of culture medium (G1®, Vitrolife, Göteborg) covered with mineral oil (OVOIL®, Vitrolife, Göteborg) and then incubated at 37°C in 6% CO_2 and 95% RH. Fertilization was assessed by the presence of pronuclei (PN) and extrusion of two polar bodies.

Day three (D3) embryos were classified according to the Istanbul consensus Workshop on Embryo Assessment [13]. Grade 1 was defined as good quality embryos with fragmentation < 10%, 7–9 blastomeres and no multinucleation; grade 2 as fair quality embryos with 10–25% fragmentation, more than six blastomeres and no evidence of multinucleation and grade 3 as poor-quality embryos with > 25% fragmentation, less than five cells (or abnormal cell size) and evidence of multinucleation. On D3, two of the best quality embryos were transferred into the uterus or frozen,

and the rest were cultured to D5 or D6. Blastocysts reaching the expansion stage, with inner cell mass and trophectoderm layer Gardner scores of A or B [14], were frozen. On D3 or D5 1–2 optimal embryos were selected for transplantation and following transfer, women were given corpus luteum support.

Statistical analysis

Data is presented as the mean ± standard deviation (SD) for all the continuous variables whereas categorical variables were presented in percentage form. The difference of continuous variables between the study groups was evaluated by ANOVA and percentage data was evaluated by Chi-square test. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using IBM SPSS 16.0 (New York, USA) statistical software.

Normal fertilization (2PN) rate was calculated as number of 2PN zygotes/number of MII oocytes. The fertilization rate was calculated as number of multi-PN, 2PN zygotes and 1PN zygotes/number of MII oocytes. Blastocyst formation was defined as development past the early blastocyst sage. Clinical pregnancy was defined as the presence of one or more gestational sacs visualized on ultrasound two weeks after embryo transfer. CPR was based on a clinical pregnancy following the use of all fresh and frozen embryos derived from a single ovarian stimulation cycle (clinical pregnancy after embryo transfer performed between January 2016 to June 2020). The implantation rate was calculated as number of gestational sacs/number of embryos transferred; cumulative ongoing pregnancy was defined as cumulative pregnancy with a detectable heart rate after 12 weeks of gestation, CLBR was defined as the first live birth following the use of all fresh and frozen embryos derived from a single ovarian stimulation cycle (live birth after embryo transfer performed between January 2016 to June 2019).

RESULTS

Baseline characteristics of the patients and sperm incubation groups

Patients (n = 1453) were divided into five SI groups, namely ≤ 1 h, 1–2 h, 2–3 h, 3–4 h or > 4 h. The baseline characteristics and sperm parameters for each SI group are summarized in Table 1. For the female, age, BMI, dose and days of gonadotrophin stimulation and days of gonadotrophin showed no statistical difference between each SI group. For the male, age and sperm volume, concentration and morphology also showed no statistical differences between the SI groups. However, the mean number of MII oocytes and D3 good quality embryos per cycle were significantly fewer in the ≤ 1h and > 4 h SI groups.

Fertilization rate and embryo development in the SI groups

There was a significant correlation between the SI time for fertilization and embryo development outcomes (Tab. 2). The fertilization rate for ≤ 1 h and > 4 h SI groups was significantly lower ($p < 0.05$) than other SI groups. The 2PN rate of > 4 h SI group was also significantly lower than other SI groups ($p < 0.01$). The blastocyst rate of > 4 h SI group was significantly lower than 2–3 h SI group (45.5% vs 56.1%, $p < 0.05$); and 1–2 h SI group also had significant difference with 2–3 h and 3–4 h SI group (48.9% vs 56.1% and 54.6%, $p < 0.05$). However, there were no difference in the percentage of D3 good quality embryos amongst the SI groups ($p > 0.05$).

The influence of sperm incubation time to the IVF treatment and birth outcomes is shown in Table 3.

Embryos from the ≤ 1 h SI group had a lowest implantation rate from fresh embryo transfer cycles (19.6%), and there was a significant difference compared with 1–2 SI group ($p < 0.001$). Among 1946 ovarian stimulation

Table 1. Baseline characteristics of the patients in the SI groups

	≤ 1 h (n = 95)	1–2 h (n = 523)	2–3 h (n = 725)	3–4 h (n = 226)	> 4 h (n = 53)	p value
Female age [years]	34.9 ± 5.3	33.8 ± 5.2	33.9 ± 5.4	33.7 ± 5.4	34.6 ± 4.9	0.319
BMI [kg/m^2]	21.0 ± 3.1	22.1 ± 3.9	22.2 ± 3.1	23.5 ± 18.4	21.6 ± 2.9	0.253
Male age [years]	37.3 ± 5.4	36.2 ± 5.9	35.9 ± 6.2	35.5 ± 6.6	35.7 ± 5.2	0.231
Sperm Volume [mL]	2.7 ± 1.2	2.8 ± 1.4	2.7 ± 1.8	3.0 ± 4.4	2.8 ± 1.2	0.836
Sperm Concentration [10^6/mL]	63.4 ± 27.9	70.7 ± 31.8	73.3 ± 33.6	72.7 ± 34.6	68.6 ± 17.6	0.114
Sperm Morphology abnormal [%]	93.5 ± 2.5	93.1 ± 2.9	93.3 ± 2.8	93.4 ± 3.0	94.4 ± 1.9	0.269
WHO sperm a [%]	39.1 ± 9.9	40.0 ± 10.6	40.9 ± 10.0	40.6 ± 10.8	40.1 ± 7.3	0.085
Total dose of gonadotrophins [IU/mL]	2859.5 ± 1156.3	2587.8 ± 1065.1	2604.2 ± 1079.4	2580.2 ± 1202.6	2733.8 ± 1549.3	0.313
Days of gonadotropin stimulation	10.9 ± 1.7	11.1 ± 1.8	11.0 ± 1.9	10.9 ± 1.9	10.6 ± 2.5	0.801
Mean No. of MII oocytes	8.1 ± 5.8^a	10.7 ± 6.3^b	10.4 ± 6.5^b	10.7 ± 6.6^b	7.3 ± 4.1^a	0.001
Mean No. of D3 good embryo	2.6^{ac}	3.3^b	3.2^{ab}	3.4^b	2.3^{ac}	0.035

Data are presented as mean ± SD; WHO — World Health Organization

Table 2. Fertilization rate and embryo development in the SI groups

	≤ 1 h	1–2 h	2–3 h	3–4 h	> 4 h	total
FR	90.6% (693/765)[a]	92.6% (5182/5594)[b]	91.9% (6956/7567)[ba]	92.3% (2231/2418)[ba]	85.5% (389/455)[c]	92.0% (15451/16799)
2PN rate	82.9% (634/765)[a]	82.3% (4603/5594)[a]	82.5% (6239/7567)[a]	82.8% (2001/2418)[a]	75.4% (343/455)[b]	82.3% (13820/16799)
D3 good embryo rate	40.5% (250/617)[a]	39.5% (1768/4477)[a]	38.4% (2341/6095)[a]	40.0% (776/1940)[a]	36.4% (122/335)[a]	39.0% (5257/13464)
Blastocyst formation rate	55.2% (116/210)[a]	48.9% (796/1629)[ab]	56.1% (1245/2220)[ac]	54.6% (341/625)[acd]	45.5% (45/99)[abd]	53.2% (2543/4783)

FR — fertilization rate; FR: [a–b] — P<0.05 — [a–c] — $p < 0.01$, [b–c] — $p < 0.01$; 2PN rate: [a–b] — $p < 0.01$; blastocyst formation rate: [b–c] — $p < 0.05$, [c–d] — $p < 0.05$, [b–d] — $p < 0.05$

Table 3. The influence of sperm incubation time to the IVF treatment and birth outcomes

	≤ 1 h	1–2 h	2–3 h	3–4 h	> 4 h	Total
IR	19.6% (11/56)[a]	38.0% (89/234)[b]	35.0% (131/374)[ab]	34.2% (39/114)[ab]	33.3% (5/15)[ab]	34.7% (275/793)
Fresh ET /Frozen ET	25/68[a]	123/509[a]	195/700[a]	60/212[a]	8/45[a]	411/1535
D3ET/D5ET	78/15[a]	509/123[a]	722/173[a]	234/38[a]	43/10[a]	1586/360
Mean cycles of ET	1.24[a]	1.38[a]	1.39[a]	1.35[a]	1.29[a]	1.37
Single embryo/ two embryo ET	16/77[a]	99/533[a]	151/744[a]	34/238[a]	6/47[a]	307/1639
CPR	62.7% (47/75)[a]	73.7% (337/457)[b]	67.7% (435/643)[a]	72.1% (145/201)[ab]	68.3% (28/41)[ab]	70.0% (992/1417)
COPR	53.3% (40/75)[a]	65.0% (297/457)[a]	59.4% (382/643)[a]	63.2% (127/201)[a]	51.2% (21/41)[a]	61.2% (867/1417)
CLBR	45.6% (31/68)[a]	63.2% (265/419)[b]	56.7% (337//594)[ac]	61.2% (112/183)[bcd]	52.8% (19/36)[abcd]	52.8% (764/1300)
sex ratio (male: female)	0.76 (16:21)	1.06 (169：159)	0.92 (201：218)	1 (76:76)	1.46 (16:11)	0.99 (478:485)
Fetus malformation rate	0% (0/37)	0.3% (1/328）	0.95%（4/421）	0.7%（1/152）	0%（0/27）	0.6% (6/965)

CPR — cumulative pregnancy rate; COPR — cumulative ongoing pregnancy rate; CLBR — cumulative live birth rate; IR: [a–b] — $p < 0.01$; CPR: [a–b] — $p < 0.05$; COPR: [a–b] — $p < 0.05$; CLBR: [a–b] — $p < 0.01$, [b–c] — $p < 0.05$, [a–d] — $p < 0.05$, [c–d] — $p > 0.05$

cycles resulting in embryo transfers (fresh or/and frozen embryo transfer), 998 had one ET, 332 had 2 ETs, 75 has three ETs and 14 had more than three ETs. There were no differences in the mean embryo transfer cycles, ratio of single and two embryos transfer cycles and ratio of D3 cleavage embryo and D5 blastocyst embryo transfers among SI groups; however, the ≤ 1h SI group had the lowest cumulative clinical outcomes. The CPR and COPR were not significantly different among the five SI groups. However, ≤ 1h SI group also have the lowest cumulative live birth rate (CLBR) (44.0%), it had statistic differences with 1–2 SI group and 3–4 SI group (45.6% vs 63.2%, $p < 0.01$; 45.6% vs 61.2%, $p < 0.05$). There were no significant differences for newborn health and gender ratio outcomes between the five SI groups.

DISCUSSION

In this retrospective study, we investigated the relationship between sperm incubation time and IVF outcomes. Our results showed that different preincubation time at 37°C 6% CO_2 for processed sperm before insemination can affect the fertilization rate, the 2PN rate, the blastocyst formation rate and embryo implantation rate in IVF. In addition, there were some differences in cumulative clinical outcomes such as cumulative pregnancy, cumulative ongoing pregnancy and cumulative live birth. For achieving improved IVF outcomes, our findings suggest that the optimal preincubation sperm time is one to four hours before insemination.

We separated the IVF cycles for five groups as ≤ 1 h, 1–2 h, 2–3 h, 3–4 h and > 4 h SI groups, and analyzed the effect of different incubation time on IVF fertilization rate. The results showed a significant decrease in fertilization rate and 2PN rate with > 4h SI time ($p < 0.01$), these results could be explained by two conclusions proved before, the sperm motility and viability decline rapidly when spermatozoa were incubated at 37°C for more than four hours [4]. The fertilization rate will decrease with the sperm loss of motion [15]; and high sperm DNA fragmentation has a significant negative association with fertilization rate [16], more than four hour incubation of sperm will increase the DNA fragmentation [8, 9, 17].

The results also showed a significant decrease in fertilization rate with ≤ 1 h SI time ($p < 0.05$). this is a phenomenon we have not noticed before. However, try to look on the time course of sperm capacitation and AR, we could find the

seasonality of this result. Prior to interaction with the oocyte, spermatozoa must undergo capacitation, then become able to undergo the acrosome reaction and to develop hyperactivation, these two capacitation-associated events will allow sperm to pass through the cumulus oophorous that surround the egg, to bind to and penetrate the zona pellucida, and finally to fuse with the egg plasma membrane. A series events happened during sperm capacitation, many key events are time-dependent, for example, the superoxide production of human spermatozoa started immediately at the beginning of incubation under capacitating, reached a plateau 15–25min later, and was sustained for > 4 h [18]. Early events during capacitation are the production of ROS and activation of the PKA pathway, the PKA activity is maximal at 30 min of capacitation in human sperm [19– 21]. Sperm hyperactivation peaked after one to three hours of incubation [18] and the phosphorylation significantly increased 1h after the beginning of the incubation, which play a role in the control of sperm motility [22]. There is also an increase in the SH content of Triton X-100 detergent--soluble proteins, which is time-dependent occurring during the first 30–60 min of capacitation [23, 24]. Therefore, it is possible that the sperm preincubation time less than one hour would be insufficient for capacitation.

We also analyzed the effect of sperm SI time on D3 embryo quality and blastocyst formation. > 4h SI time has effect on blastocyst formation rate, compared with 2–3 h SI group, the difference was significantly ($p < 0.05$), whereas there was no such effect on D3 embryo quality. Whether embryo quality in IVF cycles negatively correlated with sperm DNA fragmentation, however there is a controversy, Xue et al. [25], did not find DNA fragmentation affecting the effectiveness of IVF, however, several other studies confirmed sperm DNA fragmentation does have negative effect [16, 26–28]. If sperm incubation time truly associated with the increasing of DNA fragmentation, our results will support the conclusion of sperm DNA fragmentation have negative effect to embryo development and blastocyst formation in IVF.

In addition, we evaluated the effect of sperm on IVF outcome with different preincubation time. The results showed that shorter than one hour SI significantly effect IR in fresh embryo transfer cycles after IVF, the CPR and CLBR of ≤ 1 h SI group were also at the lowest level, there are no relative studies on this, one reason could be the quantity of good embryos in this SI group was significantly fewer, but it is far from adequate, we speculate that when sperms are preincubated for less than one hour, most of the optimized sperms not be stimulated enough, the competitive mode is not fully opened, some sub-optimal sperms have the opportunity to penetrate into the oocyte. These sub-optimal sperms decrease the embryos implantation ability, then decrease the CPR and CLBR, it's just a speculation, to confirm this speculation, research on changes of sperm incubation time should be further shorten to less than one hour.

There were no differences in the effect of > 4 h SI time on IR, CPR, COPR and CLBR, however, these parameters were lower among five SI groups. While the association between sperm DNA fragmentation and alterations in pregnancy or live birth rates in IVF also have contrary, a negative correlation between the intensification of DNA fragmentation and the achievement of pregnancy and live birth in IVF has been reported [16, 26–28], whereas other author reported there was no influence [29]. For the data we calculated were CPR and CLBR, and the outcomes they evaluated were pregnancy rate and live birth rate, there was an inconsistency in evaluation method. We cannot support the views of either side. However, there were a lot of early pregnancy loss in > 4 h SI group (CORP minus CPR), this was inconsistent with previous research which demonstrated there was no increased risk of early miscarriage after IVF in high sperm DNA fragment groups [29].

CONCLUSIONS

In conclusion, the findings of this study demonstrate that sperm preincubation time has effect on the fertilization, embryo quality and clinical outcome after IVF. One to four hours preincubation time for prepared normozoospermic at 37°C 6% CO_2 prior to be used should be considered as a necessary procedure in IVF. In order to keep this preincubation time, we suggest that normozoospermic samples collection could be taken three to five hours before insemination, we'd better to guide couple males collect semen post oocyte pick up, after COCs checking and insemination plan making. Further research is required into the mechanisms responsible for effecting of short sperm incubation time on IVF.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request. For more information, please contact Dr. Liu at liusai1316@163.com.

Conflict of interests

The authors declare that they have no competing interests.

Authors' contributions

Sai Liu and Yuanqing Yao designed this paper, Sai Liu, Guoxuan Wu, Yanyan Zhao, yongqing Lv and Nannan Dang collected data and performed data analysis, Sai Liu wrote this manuscript, Li Wang and Yao Yuanqing revised this manuscript, and and all authors read this paper.

REFERENCES

1. Whitaker M, Fraser LR, Weir BJ. Cell messengers at fertilization. . J Reprod Fertil. 1990; 42(Suppl): 1–254.
2. Guraya S. Cellular and molecular biology of capacitation and acrosome reaction in spermatozoa. International Review of Cytology. 2000; 199: 1–64, doi: 10.1016/s0074-7696(00)99001-6.
3. Marín-Briggiler C, Tezón J, Miranda P, et al. Effect of incubating human sperm at room temperature on capacitation-related events. Fertility and Sterility. 2002; 77(2): 252–259, doi: 10.1016/s0015-0282(01)02982-x.
4. Jeulin C, Serres C, Jouannet P. The effects of centrifugation, various synthetic media and temperature on the motility and vitality of human spermatozoa. Reprod Nutr Dev. 1982; 22(1A): 81–91, doi: 10.1051/rnd:19820107, indexed in Pubmed: 7156474.
5. Lachaud C, Tesarik J, Cañadas ML, et al. Apoptosis and necrosis in human ejaculated spermatozoa. Hum Reprod. 2004; 19(3): 607–610, doi: 10.1093/humrep/deh130, indexed in Pubmed: 14998959.
6. Scott L, Smith S. Mouse in vitro fertilization, embryo development and viability, and human sperm motility in substances used for human sperm preparation for assisted reproduction. Fertil Steril. 1997; 67(2): 372–381, doi: 10.1016/S0015-0282(97)81925-5, indexed in Pubmed: 9022617.
7. Calamera JC, Fernandez PJ, Buffone MG, et al. Effects of long-term in vitro incubation of human spermatozoa: functional parameters and catalase effect. Andrologia. 2001; 33(2): 79–86, doi: 10.1046/j.1439-0272.2001.00409.x, indexed in Pubmed: 11350371.
8. Nabi A, Khalili MA, Halvaei I, et al. Prolonged incubation of processed human spermatozoa will increase DNA fragmentation. Andrologia. 2014; 46(4): 374–379, doi: 10.1111/and.12088, indexed in Pubmed: 24689689.
9. Zhang XD, Chen MY, Gao Y, et al. The effects of different sperm preparation methods and incubation time on the sperm DNA fragmentation. Hum Fertil (Camb). 2011; 14(3): 187–191, doi: 10.3109/14647273.2011.604817, indexed in Pubmed: 21859363.
10. Mallett PJ, Stock CE, Fraser LR. Acrosome loss in human sperm incubated in vitro under capacitating conditions. Int J Androl. 1985; 8(5): 357–364, doi: 10.1111/j.1365-2605.1985.tb00848.x, indexed in Pubmed: 4093200.
11. Dolci S, Manna C, Jannini EA, et al. ATP-content and kinetics of acrosome reaction in human spermatozoa: influence of various culture media and incubation time. Andrologia. 1988; 20(2): 169–172, doi: 10.1111/j.1439-0272.1988.tb00684.x, indexed in Pubmed: 3389542.
12. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva. World Health Organization press. 2010.
13. ALPHA Scientists In Reproductive Medicine, ESHRE Special Interest Group Embryology. Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Reprod Biomed Online. 2011; 22(6): 632–646, doi: 10.1016/j.rbmo.2011.02.001, indexed in Pubmed: 21481639.
14. Gardner DK, Schoolcraft WB. In vitro culture of the human blastocyst. . In: Jansen R, Mortimer D. ed. Towards Reproductive Certainty: Infertility and gGenetics Beyond. Parthenon Publishing, Carnforth 1999: 378–388.
15. Zollner U, Martin S, Liebermann J, et al. Evaluation of a cut-off value for sperm motility after different hours of incubation to select the suitable reproductive technology (IVF or ICSI). Acta Obstet Gynecol Scand. 1999; 78(4): 326–331, indexed in Pubmed: 10203301.
16. Oleszczuk K, Giwercman A, Bungum M. Sperm chromatin structure assay in prediction of in vitro fertilization outcome. Andrology. 2016; 4(2): 290–296, doi: 10.1111/andr.12153, indexed in Pubmed: 26757265.
17. Gosálvez J, Cortés-Gutierez E, López-Fernández C, et al. Sperm deoxyribonucleic acid fragmentation dynamics in fertile donors. Fertil Steril. 2009; 92(1): 170–173, doi: 10.1016/j.fertnstert.2008.05.068, indexed in Pubmed: 18692792.
18. de Lamirande E, Gagnon C. Capacitation-associated production of superoxide anion by human spermatozoa. Free Radic Biol Med. 1995; 18(3): 487–495, doi: 10.1016/0891-5849(94)00169-k, indexed in Pubmed: 9101239.
19. Lefièvre L, Jha KN, de Lamirande E, et al. Activation of protein kinase A during human sperm capacitation and acrosome reaction. J Androl. 2002; 23(5): 709–716, indexed in Pubmed: 12185106.
20. Harrison RAP. Rapid PKA-catalysed phosphorylation of boar sperm proteins induced by the capacitating agent bicarbonate. Mol Reprod Dev. 2004; 67(3): 337–352, doi: 10.1002/mrd.20028, indexed in Pubmed: 14735495.
21. O'Flaherty C, de Lamirande E, Gagnon C. Phosphorylation of the ArginineXX(Serine/ Threonine) motile in human sperm proteins during capacitation: modulation and protein kinase A dependency. Mol Hum Reprod. 2004; 10: 355–63.
22. Leclerc P, de Lamirande E, Gagnon C. Regulation of protein-tyrosine phosphorylation and human sperm capacitation by reactive oxygen derivatives. Free Radic Biol Med. 1997; 22(4): 643–656, doi: 10.1016/s0891-5849(96)00379-6, indexed in Pubmed: 9013127.
23. de Lamirande E, Gagnon C. Paradoxical effect of reagents for sulfhydryl and disulfide groups on human sperm capacitation and superoxide production. Free Radic Biol Med. 1998; 25(7): 803–817, doi: 10.1016/s0891-5849(98)00156-7, indexed in Pubmed: 9823546.
24. de Lamirande E, Gagnon C. Redox control of changes in protein sulfhydryl levels during human sperm capacitation. Free Radic Biol Med. 2003; 35(10): 1271–1285, doi: 10.1016/s0891-5849(03)00501-x, indexed in Pubmed: 14607526.
25. Xue LT, Wang RX, He B, et al. Effect of sperm DNA fragmentation on clinical outcomes for Chinese couples undergoing in vitro fertilization or intracytoplasmic sperm injection. J Int Med Res. 2016; 44(6): 1283–1291, doi: 10.1177/0300060516664240, indexed in Pubmed: 28322098.
26. Simon L, Murphy K, Shamsi MB, et al. Paternal influence of sperm DNA integrity on early embryonic development. Hum Reprod. 2014; 29(11): 2402–2412, doi: 10.1093/humrep/deu228, indexed in Pubmed: 25205757.
27. Breznik BP, Kovačič BSc, Veljko Vlaisavljević V. Are sperm DNA fragmentation, hyperactivation, and hyaluronan- binding ability predictive for fertilization and embryo development in in vitro fertilization and intracytoplasmic sperm injection? Fertil Steril. 2013; 99(5): 1233–1241, doi: https://doi.org/10.1016/j.fertnstert.2012.11.048, indexed in Pubmed: 23290739.
28. Borges Jr E, Zanetti BF, Setti AS, et al. Sperm DNA fragmentation is correlated with poor embryo development, lower implantation rate, and higher miscarriage rate in reproductive cycles of non-male factor infertility. Ferti Steril. 2019; 112(3): 483–490, doi: 10.1016/j.fertnstert.2019.04.029 , indexed in Pubmed: 31200969.
29. Zhao J, Zhang Q, Wang YG, et al. Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization /intracytoplasmic sperm injection: a systematic review and meta-analysis. Fertil Steril. 2014; 102: 998–1005e8, doi: 10.1016/j.fertnstert.2014.06.033 , indexed in Pubmed: 25190048.

VIA MEDICA

ORIGINAL PAPER / OBSTETRICS

Ginekologia Polska
2023, vol. 94, no. 2, 113–118

ISSN 0017–0011, e-ISSN 2543–6767

DOI 10.5603/GP.a2021.0246

Elabela levels in pregnancies with intrauterine growth retardation

Gulen Yener[1], Salih Burcin Kavak[2], Yeliz Gul[3], Ebru Celik Kavak[2], Funda Gulcu Bulmus[4], Cengiz Sanli[3], Ibrahim Batmaz[5], Gulay Bulu[6]

[1]Department of Obstetrics and Gynecology, Tuzla State Hospital, Tuzla State Hospital, Tuzla, Istanbul, Turkey
[2]Department of Obstetrics and Gynecology, Firat University, School of Medicine, Elazig, Turkey
[3]Department of Obstetrics and Gynecology, Fethi Sekin City Hospital, Fethi Sekin City Hospital, Elazig, Turkey
[4]Department of Nutrition and Dietetics, Balikesir University Faculty of Health Sciences, Balikesir University, Faculty of Health Sciences, Balikesir, Turkey
[5]Department of Obstetrics and Gynecology, Hani State Hospital, Hani State Hospital, Diyarbakir, Turkey
[6]Department of Obstetrics and Gynecology, Kovancılar State Hospital, Kovancılar State Hospital, Elazig, Turkey

ABSTRACT

Objectives: The aim of our study is to examine maternal serum Elabela levels in pregnancy with intrauterine growth retardation (IUGR). IUGR is one of the most important causes of perinatal mortality and morbidity. IUGR is also related future comorobidities such as diabetes mellitus, hyperlipidemia, hypertension and coronary artery disease.

Material and methods: Fifty pregnancies diagnosed as IUGR (Group 1) and fifty healthy pregnancies (Group 2) enrolled into the study. Obstetric and demographic characteristics of the patients, serum elabela levels, ultrasound parameters, cord pH value and APGAR scores of the newborns were recorded. In the study, which was planned as a prospective case-control study, an independent t test was used for the evaluation of continuous data and the Mann Whitney U test was used for the statistical evaluation of ordinal data. $p < 0.05$ was considered significant.

Results: The mean gestational age of the cases at delivery was 36.35 ± 1.29 in Group 1 and 38.16 ± 0.94 weeks in Group 2 ($p < 0.05$). Mean serum Elabela levels were 15.05 ± 9.03 in Group 1 and 8.96 ± 4.33 ng/mL in Group 2 ($p < 0.0001$). Mean newborn weights were 2498.20 ± 465.92 in Group 1 and 3179.44 ± 387.99 gr. in Group 2 ($p < 0.0001$). Systolic and diastolic blood pressure measurements taken on the day of delivery were higher in Group 1, and diastolic blood pressure was 77.0 ± 9.53 in Group 1 and 72.60 ± 13.37 mmHg in Group 2 ($p < 0.05$). Bilateral uterine artery Pulsatile Index (PI) and umbilical artery PI value were significantly higher in Group 1 ($p < 0.05$), and middle cerebral artery PI and cerebroplacental ratio were significantly lower in Group 1 compared to Group 2 ($p < 0.05$). Although the cord pH value, 1st and 5th minute APGAR scores were lower in Group 1 compared to Group 2, no statistically significant difference was found ($p > 0.05$).

Conlusions: In our study, it was found that serum Elabela levels increased significantly in pregnancies complicated by IUGR compared to the control group.

Key words: pregnancy; intrauterine growth retardation; Elabela

Ginekologia Polska 2023; 94, 2: 113–118

INTRODUCTION

Normal fetal growth depends on maternal, fetal, placental, and external factors as well as genetic growth potential. Disruption in one or more of these factors may affect fetal growth and lead to intrauterine growth retardation (IUGR) [1]. IUGR, which is often defined as birth weight below the 10th percentile, remains to be one of the important causes of perinatal mortality and morbidity in modern obstetric practice [2]. Perinatal mortality rate in infants with IUGR increases 10–20 times compared to that in normal infants [3]. IUGR is also closely related to postnatal morbidities, such as insecure fetal condition, perinatal asphyxia, need for prolonged stay in the neonatal intensive care unit after delivery, and hypoglycemia. In addition, there is an increased

Corresponding author:
Salih Burcin Kavak
Firat University, School of Medicine, Department of Obstetrics and Gynecology, Elazig, Turkey
e-mail: burcinkavak1@gmail.com

Received: 16.06.2021 Accepted: 28.12.2021 Early publication date: 7.03.2022

risk of coronary artery disease, type 2 diabetes mellitus, hyperlipidemia, psychiatric diseases, and hypertension in fetuses with IUGR at later ages [4].

In recent years, Doppler ultrasonography has become a popular imaging method in the antenatal diagnosis of IUGR, because it is both non-invasive and easily applicable. Owing to this method, the presence and severity of fetal hypoxemia can be accurately determined and a significant reduction in mortality and morbidity can be achieved with timely intervention [5]. In addition, fetal biophysical profile (BFP), nonstress test (NST), and arterial and venous Doppler ultrasonography appear to be synergistically effective in detecting fetal risk in early-onset IUGR and prolonging pregnancy safely [6].

Elabela is a placental peptide hormone that was recently discovered to be the endogenous ligand for apelin (APJ), a receptor bound to G-protein. The APJ receptor is widely expressed in several tissues of the human body. Apelin is another endogenous ligand of the APJ receptor that is of the same origin as that of Elabela. Elabela and apelin have a series of similar functions. Elabela-APJ system also plays an important role in fetal cardiovascular development. In addition, this system has shown to have important biological effects, such as embryonic development, skeletal development, angiogenesis, and vascular morphogenesis. Theoretically, Elabela could play a role in preventing preeclampsia by lowering the blood pressure and proteinuria levels during pregnancy. Also, the deficiency of Elabela may cause developmental defects in the embryo and various morbidities in pregnant women. Several studies to date have, therefore, investigated the relationship between Elabela levels during pregnancy and the development of preeclampsia, gestational diabetes mellitus, and obesity [7, 8]. However, according to our knowledge, the association between serum Elabela levels and IUGR in pregnant women has been studied in only one study.

The aim of this study is to investigate the possible relationship between Elabela levels during pregnancy and IUGR.

MATERIAL AND METHODS

This single center prospective case-control study was conducted on pregnant women who presented to the gynecology and obstetrics department for antenatal examination between 2017 and 2018. Before starting the study, approval was obtained from the local ethics committee (*Ethics Committee approval no:* 2018.09.06-14-15). Informed consent was obtained from all the study participants.

Patient selection

A total of 100 cases, including 50 cases (Group 1) diagnosed with IUGR and followed up in the Gynecology and Obstetrics Clinic, and 50 cases with normal fetal development (Group 2), were included in the study.

Determination of gestational age was made according to the last menstrual period and confirmed by ultrasonography in the first trimester. The antenatal diagnosis of IUGR was based on the fetal abdominal circumference below the 10th percentile at the 3rd trimester. Umbilical artery pulsatility index (PI), Middle Cerebral Artery (MCA) PI or cerebro-placental ratio (CPR) values were used in Doppler ultrasonography in order to differentiate the fetuses with IUGR from small for gestational age fetuses. The IUGR group (Group 1) was thereby composed of the patients with abnormal Doppler ultrasonography parameters mentioned above. (Umbilical Artery PI > 95th percentile, Cerebroplacental Ratio and MCA PI <5th percentile were considered abnormal).

Patients were excluded from the study if they had a severe physical disease, pregestational diabetes, liver and kidney failure, any endocrine disorder, hematological disease, received medical treatment for any reason in the last three months, chronic inflammation or infection, patients under 19 and over 35 years old, patients with BMI < 19 and > 30, small for gestational age fetuses, multiple pregnancies, congenital anomalies, fetuses with risk of genetic screening tests (> 1/250) and if they were smokers and drugs and alcohol abusers.

Biochemical and coagulation parameters, complete urinalysis, complete blood counts, systolic and diastolic arterial blood pressure values of the cases included in the study were recorded on the day of delivery.

Sampling and evaluation

Ten milliliters of venous blood samples were collected from all pregnant women included in the study to determine Elabela levels. The blood samples were centrifuged in the laboratory of Biochemistry Department at FU and stored at –80°C. Serum Elabela levels were analyzed using human Elabela enzyme-linked immunosorbent assay (ELISA) kit (Shanghai Sunred Biological Technology Co., Ltd, catalog no: 201-12-8569, China), according to the instruction manual. Absorbances were read spectrophotometrically at 450 nm in an ELISA microplate reader (Thermo Scientific, Multiskan FC, USA). The results were given in ng/mL. The kit sensitivity was 0.118 ng/mL, the measurement range was 0.15–40 ng/mL.

The measurement process of Elabela levels was performed on the condition that the laboratory technician was unaware of the results and the patient group.

Statistical evaluation

SPSS 21.0 program (IBM, Armonk, NY, USA) was used for statistical analysis of the data. The Kolmogorov-Smirnov test was used for normality analysis of continuous variables. An independent sample t-test was used for comparison of normally distributed continuous variables, and the Mann-

-Whitney U test was used to compare continuous variables without normal distribution. The Fischer's exact test was used to compare proportional distributions of 2 × 2 nominal variables, while a chi-square test was used for n × n variables. The level of statistical significance was set at a p-value of 0.05.

RESULTS

Body mass index (BMI), number of pregnancies, parity, abortion, and curettage numbers were similar between Group 1 and Group 2. Body mass index (BMI), number of pregnancies, parity, abortion and curettage numbers were similar between Group 1 and Group 2, and no statistical difference was found between both groups. The values are shown in Table 1. However, the gestational week at which delivery took place was found to be significantly different between both groups and the delivery occurred earlier in Group 1. (Gestational Age at birth is 36.35 ± 1.29 for Group 1 and 38.16 ± 0.94 for Group 2.). The results are shown in Table 1.

The average age of the patients was 27.86 ± 4.94 in Group 1 and 28.12 ± 3.86 in Group 2, and no statistically significant difference was observed between the two groups ($p > 0.05$, Mann Whitney U test). Umbilical cord pH**, 1st and 5th minute APGAR scores * Although it was lower in Group 1 compared to Group 2, no statistically significant difference was found ($p > 0.05$, **independent t test, *Mann Whitney U test). While there was no significant difference between Group 1 and Group 2 between the systolic arterial blood pressure values on the day of delivery, diastolic arterial blood pressure values were significantly higher in Group 1. (77.00 ± 9.53 for Group 1 and 72.60 ± 13.37 for Group 2, $p < 0.05$, Mann Whitney U test). Fetal weight in Group 1 2498.20 ± 465.92 g and 3179.44 ± 387.99g in Group 2 and there is a statistically significant difference in Group 1 ($p < 0.0001$, independent t test) (Fig. 1 and 2).

Serum Elabela values were 15.05 ± 9.03 ng/mL for Group 1 and 8.96 ± 4.33 ng/mL for Group 2, and the values were statistically significantly higher in Group 1 ($p < 0.0001$, Mann Whitney U test).

Elabela levels, maternal age, fetal weight, the 1-minute and 5-minute appearance, pulse, grimace, activity, and respiration (APGAR) scores, cord pH value, and systolic and diastolic blood pressure values of both the groups are shown in Table 2.

Table 1. Obstetric and demographic characteristics of the patients

Parameters	Group 1 (n = 50)		Group 2 (n = 50)		p value
	Mean ±	SD	Mean ±	SD	
Gestational age [weeks]	36.35	1.29	38.16	0.94	***< 0.05****
Gravida [number]	2.46	1.69	2.32	1.46	*NS**
Parity [number]	1.13	1.32	1.43	1.67	*NS**
Abortus [number]	0.30	0.70	0.03	0.18	*NS**
Curettage [number]	0.06	0.18	0.01	0.10	*NS**
BMI [kg/m^2]	24.1	1.10	23.7	1.04	*NS***

* — Mann Whitney U test, ** — independent t test; BMI — body mass index; mean ± SD — mean ± standard deviation; NS — not nignificant

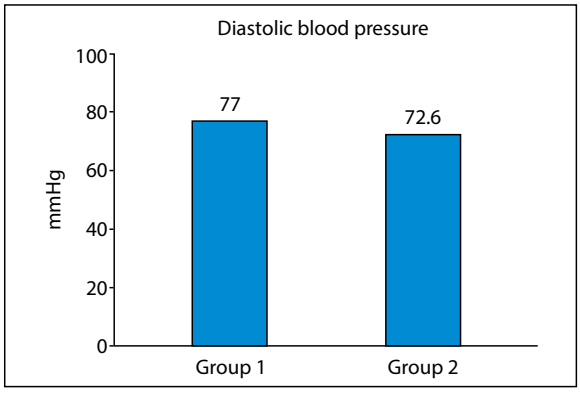


Figure 1. Diastolic blood pressure in Group 1 and Group 2

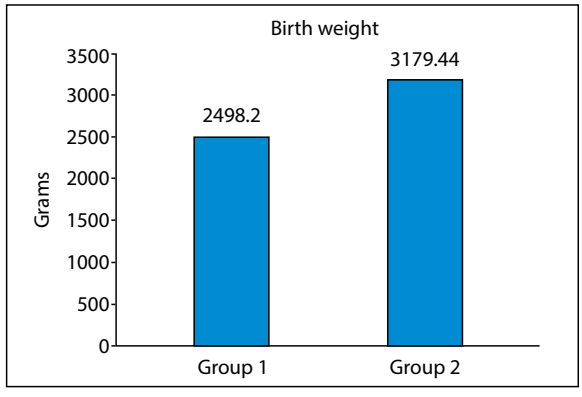


Figure 2. Birth weight in Group 1 and Group 2

Table 2. Distribution of maternal and neonatal parameters by groups

Parameters	Group 1 (n = 50)		Group 2 (n = 50)		p value
	Mean ±	SD	Mean ±	SD	
Maternal age [years]	27.86	4.94	28.12	3.86	*NS**
Elabela [ng/mL]	15.05	9.03	8.96	4.33	***< 0.0001***
NB weight [g]	2498.20	465.92	3179.44	387.99	***< 0.0001***
APGAR 1	7.12	1.10	7.36	0.78	*NS**
APGAR 5	8.84	1.09	9.16	0.68	*NS**
Cord pH	7.30	0.07	7.32	0.04	*NS**
Systolic BP [mmHg]	116.80	10.96	113.80	7.80	*NS***
Diastolic BP [mmHg]	77.00	9.53	72.60	13.37	***< 0.05****

* — Mann Whitney U test; ** — independent t test; APGAR — appearance, pulse, grimace, activity, and respiration (APGAR) Score; BP— blood pressure; mean ± SD — mean ± standard deviation; NB — Newborn; NS — not significant

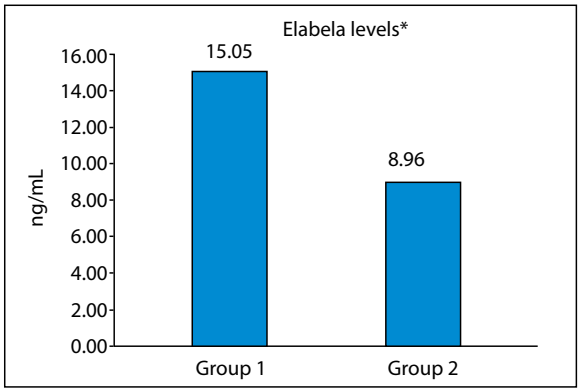


Figure 3. Serum Elabela levels in Group 1 and Group 2

Elabela levels were significantly higher in Group 1, and the p value was < 0.0001. Elabela levels of both groups are shown in Figure 3.

The cases were also examined in terms of doppler ultrasonography parameters, and all of the umbilical artery, Middle Cerebral Artery (MCA), bilateral uterine artery Pulsatility Index (PI) values and cerebroplacental ratio (CPR) measurements were found to be significantly different in Group 1 compared to Group 2 ($p < 0.05$, Mann Whitney U test). Doppler ultrasonography parameters are shown in Table 3.

DISCUSSION

Fetuses with IUGR are at high risk in terms of poor perinatal outcomes and long-term risks compared to fetuses with normal growth. The best results are obtained with the combined use of fetal biometry, biophysical profile, NST, and arterial and venous Doppler ultrasonography in follow-ups to confirm fetal well-being. The use of these tests alone has limited value in the management of IUGR. The timing of delivery in a fetus with preterm IUGR is very critical and still controversial. Gestational age is an independent factor for neonatal outcomes, and delayed delivery may increase the risk of stillbirths [9].

Studies have found that APJ or Elabela deficiency manifests as vascular defects in animals [10–12]. In contrast, apelin-deficient mice are viable and fertile; however, they show delayed retinal and cardiac vascularizations at birth [12, 13]. Consistent with this finding, Cekmez et al. showed that preterm neonates with retinopathy had lower cord blood apelin levels than preterm neonates without retinopathy [14]. The APJ receptor is highly expressed in both the endothelial precursor cells (angioblasts) and endothelial cells of the developing vasculature in the animal embryos [15, 16].

The placenta of preeclamptic women is characterized by weak trophoblastic invasion and endothelial vasospasm.

Table 3. Doppler ultrasonography parameters of the cases.

Doppler parameters	Group 1 (n = 50)		Group 2 (n = 50)		p value
	Mean ±	SD	Mean ±	SD	
Right Ut. A. PI	1.32	0.44	0.91	0.37	***< 0.05****
Left Ut. A. PI	1.12	0.32	0.75	0.17	***< 0.05****
MCA PI	1.37	0.17	1.56	0.28	***< 0.05****
Umbilical A. PI	1.17	0.07	0.80	0.11	***< 0.05****
CPR	0.91	0.19	1.90	0.28	***< 0.05****

* — Mann Whitney U test; CPR — cerebroplacental ratio; MCA PI — middle cerebral artery pulsatile index; Mean ± SD — mean ± standard deviation; Umbilical A. PI — umbilical artery pulsatile index; Ut. A. PI — uterine artery pulsatile index

These defects are considered to be the driving forces of the development of preeclampsia [17]. Proper invasion of the spiral arteries by trophoblasts during implantation relies on a good balance between placental angiogenic and antiangiogenic factors. To date, the development of preeclampsia has been associated with the elevation of two placental antiangiogenic factors, soluble Fms-like tyrosine kinase 1 (sFlt1 or sVEGFR-1) and endoglin. Indeed, application of these factors to pregnant rats reproduces preeclampsia-like symptoms [18]. Apelin controls the vascular tone in the placenta. Therefore, several studies have investigated placental APJ and apelin expression in patients with preeclampsia. Among all available studies, some studies used control and preeclampsia patient groups that did not match in terms of age, gestational age, or BMI [19, 20], or included a very small number of patients [21]. These limitations may lead to potential bias, as apelin levels can vary based on these factors [21, 22].

As mentioned earlier, pregnant mice carrying Elabela-deficient embryos show features of placental insufficiency of vascular origin and preeclampsia (hypertension, proteinuria, and glomerular endotheliosis). In addition, subcutaneous injection of Elabela to Elabela-null mice between E11 and E19 has prevented the development of maternal hypertension and proteinuria [23]. This reveals the primary role of Elabela in preeclampsia. Three studies recently measured circulating Elabela levels in preeclamptic women to evaluate whether Elabela is involved in the etiology of preeclampsia in humans. These studies revealed no difference in circulating Elabela levels between preeclampsia and control patients, except for a group of women with late-onset preeclampsia [7, 24, 25].

To date, these studies do not support the hypothesis that human preeclampsia is characterized by an early deficiency in circulating Elabela levels. In two studies using the same ELISA kit, very different Elabela levels were found in samples collected over a similar time period. Further studies are needed to establish guidelines for the adequate measurement of Elabela as well as determining the relative variation of specific Elabela isoforms.

Current literature highlights the critical role of Elabela/apelin (APJ) axis in fetal and placental development. While Elabela appears to have specific roles in early fetal development, particularly for the cardiovascular system formation, apelin function emerges afterwards to control fetal angiogenesis and energy homeostasis. Similarly, Elabela is essential for the early placental development (contributing to trophoblastic invasion and angiogenic sprouting process) [7, 23]. On the other hand, apelin regulates constitutive functions, such as placental vessel tone and nutrient exchange. Both hormones act through a common receptor expressed on multiple cell types in the fetus and placenta throughout the pregnancy [19].

PE and IUGR cause abnormal placentation, and results in adverse pregnancy outcomes [26]. Both diseases have heterogeneous etiology and risk factors are similar [27, 28]. More importantly, the histopathological features of PE and IUGR are similar [29]. The two diseases sometimes coexist. This may be due to the similarity of the pathophysiological mechanism. Further studies are needed in this area.

In the literature, there is only one study examining the maternal serum Elabela levels in cases with IUGR fetuses. In the study by Behram et al., serum Elabela levels were found to be significantly lower in IUGR cases compared to the healthy control group [30]. In the study, measurement of serum Elabela levels was performed at the 30th gestational week in both groups due to matching the gestational age. The IUGR group gave birth two weeks after the measurement, and the pregnancy continued for another 8 weeks in the control group. In addition, IUGR cases included in the study were cases with EFW below the 3rd percentile. Although these cases are more homogeneous, they are all in high-risk in terms of adverse perinatal outcomes [31]. It is possible that some patients gave birth in the second trimester due to impaired placental adaptation. In our study, cases with fetal AC measurement below the 10th percentile in the third trimester were accepted as IUGR. In our study group, different mechanisms may have been activated in terms of pregnancy adaptation and increased Elabela levels. In addition, serum Elabela levels were measured on the day of delivery in our study. The difference in Elabela levels between the two studies may also be related to this.

In addition, the results of various studies comparing preeclampsia and Elabela levels at similar weeks of gestation show conflicting results. In the study of Deniz et al, maternal serum Elabela levels were found to be significantly lower in preeclampsia and severe preeclampsia cases compared to the control group, while Elabela levels were found to be higher in preeclampsia cases in the study of Panaitescu et al. [7, 32]. No significant difference was found in the study of Pritchard et al. [25]. These results may also be a result of the complex nature of placental pathology. Similar conflicting results are likely to occur in IUGR cases.

Studies in the current literature have shown that the uterine artery PI is increased, and the middle cerebral artery PI and CPR values are decreased in pregnancies with growth retardations [33, 34]. The results of our study also support these studies.

To our knowledge, there are only few studies in the literature evaluating the diastolic arterial blood pressure in pregnancies with intrauterine growth retardations and comparing them with normal pregnancies. However,

in our study, a significant difference was found between both the groups.

CONCLUSIONS

In our study, Elabela levels were found to be 15.05 in pregnancies with IUGR. The differences in the control group were found to be significant. In this respect, we consider that placental Elabela levels increase in cases of IUGR, and this may be a protective mechanism. Comprehensive studies are needed in this area.

Conflicts of interest

All authors declare no conflict of interest.

REFERENCES

1. Gardosi J, Chang A, Kalyan B, et al. Customised antenatal growth charts. Lancet. 1992; 339(8788): 283–287, doi: 10.1016/0140-6736(92)91342-6, indexed in Pubmed: 1346292.
2. ACOG Practice Bulletin No. 204 Summary: Fetal Growth Restriction. Obstet Gynecol. 2019; 133(2): 390–392, doi: 10.1097/AOG.0000000000003071, indexed in Pubmed: 30681535.
3. Regev R, Lusky A, Dolfin T, et al. Excess mortality and morbidity among small-for-gestational-age premature infants: a population-based study. The Journal of Pediatrics. 2003; 143(2): 186–191, doi: 10.1067/s0022-3476(03)00181-1.
4. Barker DJP, Eriksson JG, Forsén T, et al. Fetal origins of adult disease: strength of effects and biological basis. Int J Epidemiol. 2002; 31(6): 1235–1239, doi: 10.1093/ije/31.6.1235, indexed in Pubmed: 12540728.
5. Alfirevic Z, Neilson JP. Doppler ultrasonography in high-risk pregnancies: systematic review with meta-analysis. Am J Obstet Gynecol. 1995; 172(5): 1379–1387, doi: 10.1016/0002-9378(95)90466-2, indexed in Pubmed: 7755042.
6. Turan S, Turan OM, Berg C, et al. Computerized fetal heart rate analysis, Doppler ultrasound and biophysical profile score in the prediction of acid-base status of growth-restricted fetuses. Ultrasound Obstet Gynecol. 2007; 30(5): 750–756, doi: 10.1002/uog.4101, indexed in Pubmed: 17688309.
7. Panaitescu B, Romero R, Gomez-Lopez N, et al. ELABELA plasma concentrations are increased in women with late-onset preeclampsia. J Matern Fetal Neonatal Med. 2020; 33(1): 5–15, doi: 10.1080/14767058.2018.1484089, indexed in Pubmed: 29890874.
8. Akcılar R, Turgut S, Caner V, et al. The effects of apelin treatment on a rat model of type 2 diabetes. Adv Med Sci. 2015; 60(1): 94–100, doi: 10.1016/j.advms.2014.11.001, indexed in Pubmed: 25625368.
9. A randomised trial of timed delivery for the compromised preterm fetus: short term outcomes and Bayesian interpretation. BJOG: An International Journal of Obstetrics and Gynaecology. 2003; 110(1): 27–32, doi: 10.1016/s1470-0328(02)02514-4.
10. Kang Y, Kim J, Anderson JP, et al. Apelin-APJ signaling is a critical regulator of endothelial MEF2 activation in cardiovascular development. Circ Res. 2013; 113(1): 22–31, doi: 10.1161/CIRCRESAHA.113.301324, indexed in Pubmed: 23603510.
11. Scott IC, Masri B, D'Amico LA, et al. The g protein-coupled receptor agtrl1b regulates early development of myocardial progenitors. Dev Cell. 2007; 12(3): 403–413, doi: 10.1016/j.devcel.2007.01.012, indexed in Pubmed: 17336906.
12. Zeng XXI, Wilm TP, Sepich DS, et al. Apelin and its receptor control heart field formation during zebrafish gastrulation. Dev Cell. 2007; 12(3): 391–402, doi: 10.1016/j.devcel.2007.01.011, indexed in Pubmed: 17336905.
13. Kasai A, Shintani N, Kato H, et al. Retardation of retinal vascular development in apelin-deficient mice. Arterioscler Thromb Vasc Biol. 2008; 28(10): 1717–1722, doi: 10.1161/ATVBAHA.108.163402, indexed in Pubmed: 18599802.
14. Cekmez F, Pirgon O, Aydemir G, et al. Correlation between cord blood apelin and IGF-1 levels in retinopathy of prematurity. Biomark Med. 2012; 6(6): 821–825, doi: 10.2217/bmm.12.82, indexed in Pubmed: 23227848.
15. Devic E, Rizzoti K, Bodin S, et al. Amino acid sequence and embryonic expression of msr/apj, the mouse homolog of Xenopus X-msr and human APJ. Mech Dev. 1999; 84(1-2): 199–203, doi: 10.1016/s0925-4773(99)00081-7, indexed in Pubmed: 10473142.
16. Helker C, Schuermann A, Pollmann C, et al. The hormonal peptide Elabela guides angioblasts to the midline during vasculogenesis. eLife. 2015; 4, doi: 10.7554/elife.06726.
17. Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med. 2004; 350(7): 672–683, doi: 10.1056/NEJMoa031884, indexed in Pubmed: 14764923.
18. Levine RJ, Lam C, Qian C, et al. CPEP Study Group. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med. 2006; 355(10): 992–1005, doi: 10.1056/NEJMoa055352, indexed in Pubmed: 16957146.
19. Yamaleyeva LM, Chappell MC, Brosnihan KB, et al. Downregulation of apelin in the human placental chorionic villi from preeclamptic pregnancies. Am J Physiol Endocrinol Metab. 2015; 309(10): E852–E860, doi: 10.1152/ajpendo.00272.2015, indexed in Pubmed: 26394665.
20. Colcimen N, Bulut G, Ergul Erkec O, et al. Investigation of role of vascular endothelial growth factor, Annexin A5 and Apelin by immunohistochemistry method in placenta of preeclampsia patients. Cell Mol Biol (Noisy-le-grand). 2017; 63(11): 42–45, doi: 10.14715/cmb/2017.63.11.8, indexed in Pubmed: 29208172.
21. Van Mieghem T, Doherty A, Baczyk D, et al. Apelin in normal pregnancy and pregnancies complicated by placental insufficiency. Reprod Sci. 2016; 23(8): 1037–1043, doi: 10.1177/1933719116630422, indexed in Pubmed: 26880769.
22. Wysocka MB, Pietraszek-Gremplewicz K, Nowak D. The Role of Apelin in Cardiovascular Diseases, Obesity and Cancer. Front Physiol. 2018; 9: 557, doi: 10.3389/fphys.2018.00557, indexed in Pubmed: 29875677.
23. Ho L, van Dijk M, Chye ST, et al. ELABELA deficiency promotes preeclampsia and cardiovascular malformations in mice. Science. 2017; 357(6352): 707–713, doi: 10.1126/science.aam6607, indexed in Pubmed: 28663440.
24. Villie P, Lefevre G, Arrestier R, et al. ELABELA concentration is not decreased in maternal plasma before the onset of preeclampsia. Am J Obstet Gynecol. 2019; 220(3): 284–285, doi: 10.1016/j.ajog.2018.12.020, indexed in Pubmed: 30576665.
25. Pritchard N, Kaitu'u-Lino TJ, Gong S, et al. ELABELA/APELA Levels Are Not Decreased in the Maternal Circulation or Placenta among Women with Preeclampsia. Am J Pathol. 2018; 188(8): 1749–1753, doi: 10.1016/j.ajpath.2018.04.008, indexed in Pubmed: 29803833.
26. Longtine MS, Nelson DM. Placental dysfunction and fetal programming: the importance of placental size, shape, histopathology, and molecular composition. Semin Reprod Med. 2011; 29(3): 187–196, doi: 10.1055/s-0031-1275515, indexed in Pubmed: 21710395.
27. Uzan J, Carbonnel M, Piconne O, et al. Pre-eclampsia: pathophysiology, diagnosis, and management. Vasc Health Risk Manag. 2011; 7: 467–474, doi: 10.2147/VHRM.S20181, indexed in Pubmed: 21822394.
28. Figueras F, Gardosi J. Intrauterine growth restriction: new concepts in antenatal surveillance, diagnosis, and management. Am J Obstet Gynecol. 2011; 204(4): 288–300, doi: 10.1016/j.ajog.2010.08.055, indexed in Pubmed: 21215383.
29. Roberts DJ, Post MD. The placenta in pre-eclampsia and intrauterine growth restriction. J Clin Pathol. 2008; 61(12): 1254–1260, doi: 10.1136/jcp.2008.055236, indexed in Pubmed: 18641412.
30. Behram M, Oğlak SC, Dağ İ. Circulating levels of Elabela in pregnant women complicated with intrauterine growth restriction. J Gynecol Obstet Hum Reprod. 2021; 50(8): 102127, doi: 10.1016/j.jogoh.2021.102127, indexed in Pubmed: 33781971.
31. Flood K, Unterscheider J, Daly S, et al. The role of brain sparing in the prediction of adverse outcomes in intrauterine growth restriction: results of the multicenter PORTO Study. Am J Obstet Gynecol. 2014; 211(3): 288.e1–288.e5, doi: 10.1016/j.ajog.2014.05.008, indexed in Pubmed: 24813969.
32. Deniz R, Baykus Y, Ustebay S, et al. Evaluation of elabela, apelin and nitric oxide findings in maternal blood of normal pregnant women, pregnant women with pre-eclampsia, severe pre-eclampsia and umbilical arteries and venules of newborns. J Obstet Gynaecol. 2019; 39(7): 907–912, doi: 10.1080/01443615.2019.1572727, indexed in Pubmed: 31064239.
33. Hart JM, Young BC. Strip of the month: intrauterine growth restriction with abnormal umbilical artery doppler studies. Neoreviews. 2019; 20(3): e161–e169, doi: 10.1542/neo.20-3-e161, indexed in Pubmed: 31261056.
34. Novac MV, Iliescu DG, Tudorache S, et al. Ultrasound evaluation of fetal biometry and doppler parameters in the third trimester of pregnancy suspected of intrauterine growth restriction. . Curr Health Sci J . 2018; 44(1): 23–28, doi: 10.12865/CHSJ.44.01.04, indexed in Pubmed: 30622751.

VIA MEDICA

ORIGINAL PAPER / OBSTETRICS

Ginekologia Polska
2023, vol. 94, no. 2, 119–128

ISSN 0017–0011, e-ISSN 2543–6767

DOI 10.5603/GP.a2021.0249

Impact of gestational diabetes and other maternal factors on neonatal body composition in the first week of life: a case-control study

Karolina Karcz, Matylda Czosnykowska-Lukacka, Barbara Krolak-Olejnik

Department and Clinic of Neonatology, Wroclaw Medical University, Poland

ABSTRACT

Objectives: Newborns of diabetic mothers are at increased risk of abnormal nutritional status at birth, thus developing metabolic disorders. The aim of this study was to evaluate the anthropometric measurements and body composition of newborns born to mothers with gestational diabetes in comparison to newborns born to mothers with normal glucose tolerance in pregnancy, in the first week of their life. Maternal factors affecting the gestational period were also evaluated.

Material and methods: The study included 70 participants: neonates born to mothers with gestational diabetes (GDM) and neonates born to healthy mothers (non-GDM). A set of statistical methods (*e.g.*, ANOVA, Kruskal-Wallis test, Chi-square test, regression, cluster analysis) was used to compare data between the study groups and to find their association with maternal factors.

Results: Our approach resulted in statistically significant classification ($p < 0.05$) by maternal history of hypothyroidism, weight gain during pregnancy and diagnosis of GDM. Newborns of mothers diagnosed with both GDM and hypothyroidism had lower birth weight and fat mass than newborns of mothers without GDM nor hypothyroidism ($p < 0.05$), however this finding might be associated with high incidence of excessive gestational weight gain among healthy mothers. No differences in body composition were found between the study groups on account of maternal GDM only ($p > 0.05$).

Conclusions: Thus, well-controlled gestational diabetes mellitus as an individual factor does not significantly affect neonatal anthropometric measurements and body composition.

Key words: gestational diabetes; hypothyroidism; body composition; newborn; bioelectrical impedance

Ginekologia Polska 2023; 94, 2: 119–128

INTRODUCTION

Based on the theory of nutritional programming, a child's nutritional status in the first 1,000 days after conception has a significant impact on the neurological development, mental health throughout the life, and the risk of developing obesity, hypertension and diabetes [1, 2]. During this period, especially in prenatal life, nutritional programming largely depends on the quality of the mother's diet and her comorbidities, which affect the supply of nutrients for the fetus [1]. Moreover, events in prenatal life (*e.g.*, maternal comorbidities or nutritional status, maternal stress) altogether with genetic and environmental factors influence the determination of a certain pattern of physiological processes (Barker hypothesis) resulting in long-term adaptive changes in the developing fetus. These adaptive changes are initially favorable, because they adapt the fetus to cover the current needs, however they can have a detrimental effect in the long-term and enhance the risk of development of non-communicable diseases in the adulthood [2].

It has conclusively been shown that disturbances in the physical development of the fetus and an increased risk of postnatal metabolic complications constitute a typical clinical picture of an infant of a diabetic mother. Currently, it is estimated that gestational diabetes affects approximately 5.4% of women in Europe and 3.4% of women in Poland [3].

The newborns of diabetic mothers are observed with increased incidence of macrosomia, polyhydramnios, stillbirths, perinatal injuries and surgical deliveries. In the long term, maternal diabetes during pregnancy increases the risk of obesity, impaired glucose tolerance and diabetes in offspring, and in the case of uncontrolled diabetes, also neurological development disorders. Nevertheless, most of

Corresponding author:
Karolina Karcz
Department and Clinic of Neonatology, Wroclaw Medical University, 213 Borowska St, 50-556 Wroclaw, Poland
e-mail: karolina.karcz@student.umed.wroc.pl

Received: 4.12.2021 Accepted: 30.12.2021 Early publication date: 4.03.2022

the above-mentioned complications result from overnutrition and fetal macrosomia, the primary source of which are maternal disorders of glucose and fat metabolism during pregnancy. Therefore, gestational diabetes requires special attention mainly for early diagnosis, appropriate treatment and metabolic control. Consequently, newborns born by mothers with gestational diabetes require special care and increased observation [4–6].

Body composition disturbances in early life, including the prenatal period, might play a key role in the programming of a variety of health disorders in the future, including hypertension, type 2 diabetes and obesity [6, 7]. It is known that changes in fat mass (FBM) are associated with changes in total body water volume (TBW), mainly extracellular water volume (ECW) and extracellular water to intracellular water ratio (E/I ratio). So far, it has been shown that obesity is associated with a disturbed ratio of individual water compartments in the body, which is not normalized by weight reduction, probably due to primary alteration in haemodynamics and fluid regulation [8, 9].

Objectives

The aim of this study was to evaluate the anthropometric measurements and body composition of neonates born to mothers with gestational diabetes in comparison to neonates born to mothers with normal glucose tolerance in pregnancy, in the first week of their life. In the present paper we also aimed to find maternal factors affecting the gestational period that might have influence on newborns' body composition.

MATERIAL AND METHODS

All 70 participants came from Poland (Wroclaw University Hospital) and were enrolled in prospective, observational case-control study after birth. Inclusion criteria for the case and control groups were: mother's age 18 – 45 years; delivery at term (≥ 37 + 0/7 weeks of gestation) or near term (from 35 + 0/7 to 36 + 6/7 weeks of gestation), both by vaginal delivery and by caesarean section; single pregnancy; good condition of the child after birth (vigorous, cardiovascularly and respiratorily stable neonate, who did not require assistance in transition to extrauterine life), rated > 7 points on the Apgar score after the 1st minute of life; exclusive or predominant breastfeeding. Exclusion criteria were any clinical condition of the mother and/or the newborn that may negatively affect the nutritional status of the newborn (IUGR, lack of medical care during pregnancy, maternal addictions to alcohol or other psychoactive substances, nicotinism in pregnancy); uncontrolled asthma in the mother; metabolic diseases in the mother or newborn).

The results of assessment of body composition and anthropometric measurements of the newborn, clinical data on the course of pregnancy and maternal pregestational medical history, childbirth, puerperium (interview from mother) in the period of postnatal hospitalization of the newborn in the Department of Neonatology, before discharge from the hospital (up to 7 days of age) were collected.

Maternal body weight changes during the pregnancy were analysed based on medical documentation. As gestational weight gain guidelines, that are based on prepregnancy body mass index (BMI), ranges for underweight, normal weight, overweight, and obese women, the categories of maternal gestational weight gain (below, within or above recommendations) were set in reference to American College of Obstetricians and Gynecologists Committee Opinion, that was approved by Polish Society of Gynaecologists and Obstetricians [3, 10].

Criteria for diagnosis of gestational diabetes (according to World Health Organization and American Diabetes Association, adopted by Polish Society of Gynaecologists and Obstetricians) based on Oral Glucose Tolerance Test (OGTT) with the use of 75 g of glucose state as follows and only one of them is enough to meet: 1) fasting blood glucose 92–125 mg/dL, 2) glycemia in 1 h OGTT ≥ 180 mg/dL, 3) blood glucose level in 2 h OGTT 153–199 mg/dL [3, 11, 12].

Management of maternal thyroid disorders and hypertension during pregnancy was consistent with international guidelines and recommendations, adopted in Poland [13–15]

Study groups

The 70 participants were being enrolled from December 2019 to February 2021. Study group was divided into 50 neonates born to mothers with Gestational Diabetes, treated with diet (GDM G1) or treated with insulin (GDM G2). The control group included 20 randomly assigned neonates of healthy non-diabetic mothers (non-GDM), born at similar gestational age, who met the eligibility criteria.

Based on the medical documentation and interview, none of the 70 mothers were diagnosed with chronic pregestational diabetes nor insulin resistance before the pregnancy. All the GDM mothers received regular medical control. A total of 20 mothers were diagnosed with chronic hypothyroidism and 13 mothers were diagnosed with gestational hypothyroidism — all of them were successfully treated with levothyroxine, which resulted in TSH level ≤ 2.5 mIU/L. Considering hypertension, it was chronic in 6 mothers and pregnancy — induced in 8 mothers — all women were treated with methyldopa. Nicotinism before pregnancy was found in 22 mothers — all of them claimed to quit smoking before the conception.

Ethical issues

The study was approved by the Bioethics Committee at the Medical University in Wroclaw (No. KB 773/2019,

Figure 1. Placement of the electrodes during body bioimpedance analysis

35/2020, 407/2020). The written and informed consents were obtained from the mothers. The presented research results were carried out within the project registered in Clinical Trials (https://clinicaltrials.gov/), NCT04937348.

Anthropometric measurements

Anthropometric measurements were taken twice — after birth and just before the body composition analysis. On the day of body composition assessment, each newborn infant was weighed naked to the nearest 10 g on an electronic baby scale (RADWAG type WPT 6 / 15D). Crown-heel length (measured in recumbent position) and occipito-frontal circumference were measured to the nearest 0.5 cm by a standard disposable non-stretchy tape. The measurements taken before body composition analysis were made by the same investigator (K.K).

Body composition assessment

Neonatal body composition was evaluated using a non-invasive method of bioimpedance analysis (BIA), which determines particular body compartments based on electrical properties of human tissues [16]. As body tissues differ in electrical conductivity due to their various hydration, a low-level electrical current sent through the body during measurement is impeded and passes through tissues with various speeds. The device measures the signal, thus determines the resistance of the electrical current, estimates body water and based on equations calculates fat mass and lean mass. This method was chosen as it is easily available, portable, noninvasive and quick in use. Based on available literature, BIA appears to be an effective and reliable technique of body composition estimations as a single use method in infants and young children [17, 18].

The measurements were made with Body Composition Monitor (BCM, Fresenius Medical Care, Germany) and dedicated disposable electrodes BCM-FMC (< 25 kg). The measurements were made at 50 frequencies over a range from 5 to 1000 kHz, with amplitude of the electric current 0.8 mA. The measurements were performed in accordance with the manufacturer's instructions, by the same investigator (K.K.). During the examination the patients were undressed, lying in a supine position. Electrodes were attached at least two minutes before measurement to the dorsal sides of one hand and one foot, with two electrodes on each extremity, providing the most possible distal location and ensuring at least 2 cm distance between the electrodes. In each patient, the electrodes were placed on the right side of the body, in similar locations — as the precision and reproducibility of electrodes placement was found important [19]. The placement of electrodes applied in the study is presented in Figure 1. The body composition assessment was made during the newborn's sleep, at least 1.5 hour after the last feeding.

Statistical analysis

For computations Microsoft Excel for Office 365 (Microsoft, Redmond, WA, USA), Statistica 13.3 (StatSoft, Inc., Tulsa, OK, USA) and R version 3.6.2 (R Core Team, 2013. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.) [20–22] were used.

The data are presented as: mean and standard deviation (SD), or median and interquartile range (IQR), or number of cases and percentage, where applicable.

The level of significance in statistical analysis was set at $\alpha = 0.05$. Comparisons of demographic and clinical data between study groups were made with one-way ANOVA, Kruskal–Wallis test and Chi-square test, depending on the type of data and their distribution. Univariate regression (generalized linear model) was used to assess the impact of selected maternal factors on the studied neonatal anthropometric parameters and body composition. As the next step, a clus-

ter analysis was performed using the Marczewski-Steinhaus' taxonomic approach (M–S) [23] and the dendrogram was built. The type effect was studied using one-way ANOVA. The verification of the taxonomic method was made using expectation-maximization (E–M) algorithm [24].

RESULTS

The overall characteristics of the newborns is presented in Table 1. There were 21 newborns in GDM G1 group, 29 newborns in GDM G2 group and 20 newborns in non-GDM group. In the whole study population median of gestational age at birth was 39.0 (IQR 2.0) weeks (range 37 41 weeks), with no significant differences between the study groups [H (2, n = 70) = 3.246, p > 0.05]. Approximately 71% (50/70) of the newborns were born by the cesarean section (main indications for cesarean section were previous cesarean section and lack of progress in labour) and 55.7% were girls — there were no significant differences in terms of sex [$\chi 2$ (2, n = 70) = 0.620, p > 0.05] and mode of delivery [$\chi 2$ (2, n = 70) = 0.533, p > 0.05] between the study groups.

Table 1. Characteristics of the study population (n = 70)

	All newborns (n = 70)	GDM G1 (n = 21)	GDM G2 (n = 29)	non-GDM (n = 20)	p value
Gestational age [weeks], Median (IQR)	39.0 (2.0)	39.0 (2.0)	38.0 (1.0)	39.0 (2.0)	0.197[a]
Sex, n (%)					
Boys	28 (40.0)	7 (33.3)	12 (41.4)	9 (45.0)	0.733[c]
Girls	52 (60.0)	14 (66.7)	17 (58.6)	11 (55.0)	
Mode of delivery, n (%)					
Vaginal birth	20 (28.6)	7 (33.3)	7 (24.1)	6 (30.0)	0.766[c]
Cesarean section	50 (71.4)	14 (66.7)	22 (75.9)	14 (70.0)	
Birth weight [kg], Mean (SD)	3.45 (0.48)	3.41 (0.57)	3.34 (0.44)	3.65 (0.4)	0.079[b]
Length [cm], Mean (SD)	53.2 (2.8)	53.0 (3.5)	53.1 (2.5)	53.6 (2.3)	0.767[b]
Head circumference [cm], Mean (SD)	34.7 (1.5)	34.5 (1.6)	34.8 (1.6)	34.7 (1.6)	0.887[b]
Maternal age [years], Mean (SD)	32.7 (4.5)	33.9 (4.9)	32.1 (4.6)	32.5 (3.9)	0.363[b]
Gravidity, Median (IQR)	2.0 (1.0)	2.0 (2.0)	2.0 (1.0)	2.0 (1.0)	0.520[a]
Parity, Median (IQR)	2.0 (1.0)	1.0 (1.0)	1.0 (1.0)	2.0 (1.0)	0.395[a]
Maternal BMI before pregnancy, Median (IQR)	24.17 (6.40)	23.34 (3.48)	28.04 (6.80)	22.96 (2.83)	0.014[a]
Maternal BMI before pregnancy, n (%)					
Normal	40 (57.1)	14 (66.7)	9 (31.0)	17 (85.0)	0.004[c]
Overweight	16 (22.9)	4 (19.0)	11 (38.0)	1 (5.0)	
Obese	14 (20.0)	3 (14.3)	9 (31.0)	2 (10.0)	
Maternal weight gain during pregnancy [kg], Mean (SD)	11.5 (5.7)	10.2 (3.5)	9.2 (5.4)	16.2 (5.4)	< 0.001[b]
Maternal weight gain during pregnancy in reference to pre-gestational BMI, n (%)					
Below recommendations	18 (25.8)	7 (33.3)	9 (31.0)	2 (10.0)	0.033[c]
Within recommendations	26 (37.1)	10 (47.7)	11 (38.0)	5 (25.0)	
Above recommendations	26 (37.1)	4 (19.0)	9 (31.0)	13 (65.0)	
Maternal history of hypertension, n (%)					
Chronic (onset before the pregnancy)	6 (8.6)	1 (4.8)	5 (17.2)	0	0.478[c]
Pregnancy induced	8 (11.4)	2 (9.5)	5 (17.2)	1 (5.0)	
None	56 (80.0)	18 (85.7)	19 (65.6)	19 (95.0)	
Maternal history of hypothyroidism, n (%)					
Chronic (onset before the pregnancy)	20 (28.6)	6 (28.6)	12 (41.4)	2 (10.0)	0.175[c]
Gestational (onset during the pregnancy)	13 (18.6)	5 (23.8)	4 (13.8)	4 (20.0)	
None	37 (52.9)	10 (47.6)	13 (44.8)	14 (70.0)	
Nicotinism before pregnancy, n (%)	22 (31.4)	6 (28.6)	11 (37.9)	5 (25.0)	0.597[c]

a — Kruskal-Wallis test; b — one-way ANOVA; SD — standard deviation; IQR — interquartile range; BMI — body mass index

Table 2. Results of the anthropometric measurements and body composition analysis in newborns (n = 70)

	GDM G1 (n = 21)	GDM G2 (n = 29)	non-GDM (n = 20)	p value
Chronological age [days], Median (IQR)	3.0 (1.0)	4.0 (1.0)	3.0 (0.5)	0.253[a]
Current weight [kg], Mean (SD)	3.21 (0.53)	3.12 (0.41)	3.42 (0.35)	0.062[b]
Length [cm], Mean (SD)	53.0 (3.5)	53.1 (2.5)	53.6 (2.3)	0.767[b]
Head circumference [cm], Mean (SD)	34.5 (1.6)	34.8 (1.6)	34.7 (1.6)	0.887[b]
BMI [kg/m^2], Mean (SD)	11.36 (1.21)	11.09 (1.18)	11.93 (1.21)	0.164[b]
PI [kg/m^3], Mean (SD)	2.16 (0.27)	2.09 (0.25)	2.24 (0.28)	0.058[b]
TBW [l], Mean (SD)	2.6 (0.5)	2.6 (0.4)	2.7 (0.4)	0.360[b]
TBW%, Mean (SD)	81.2 (4.7)	82.67 (6.77)	80.31 (6.63)	0.244[b]
ECW [l], Median (IQR)	0.8 (0.3)	0.9 (0.1)	0.9 (0.2)	0.234[a]
ICW [l], Mean (SD)	1.7 (0.3)	1.7 (0.3)	1.8 (0.3)	0.355[b]
E/I, Median (IQR)	0.4 (0.1)	0.5 (0.1)	0.5 (0.1)	0.584[a]
FBM [kg], Mean (SD)	0.27 (0.1)	0.24 (0.1)	0.31 (0.1)	0.071[b]
FBM%, Mean (SD)	8.32 (2.24)	7.5 (2.55)	9.19 (2.21)	0.208[b]
FFM [kg], Mean (SD)	2.93 (0.46)	2.89 (0.36)	3.11 (0.3)	0.142[b]
FFM%, Mean (SD)	91.62 (2.29)	92.51 (2.54)	90.96 (2.56)	0.122[b]

a — Kruskal-Wallis test; b — one-way ANOVA; SD — standard deviation; IQR — interquartile range; BMI — body mass index; PI — ponderal index; TBW — total body water; ECW — extracellular water; ICW — intracellular water; E/I — extracellular/intracellular water ratio; FBM — fat mass; FFM — fat-free mass

In the whole study population mean birth weight was 3.23 (± 0.45) kg (range 2.02–4.3 kg), mean length 53.2 (± 2.7) cm (range 47–61 cm), mean head circumference 34.7 (± 1.5) cm (range 31–38 cm). Also, these anthropometric measurements taken after birth were comparable between newborns of diabetic and non-diabetic mothers [respectively birth weight $F(2, 67) = 2.633$ $p > 0.05$; length $F(2, 67) = 0.266$ $p > 0.05$; head circumference $F(2, 67) = 0.12$ $p > 0.05$]. There were no significant differences in prevalence of maternal hypothyroidism [$\chi 2$ (2, n = 70) = 6.343, $p > 0.05$], hypertension [$\chi 2$ (2, n = 70) = 3.498, $p > 0.05$] and nicotinism before pregnancy [$\chi 2$ (2, n = 70) = 1.032, $p > 0.05$] between study groups. However, the mothers differed in BMI before pregnancy [H (2, n = 70) = 8.537, $p < 0.05$], with the highest values in GDM G2 group; and weight gain during pregnancy [$F(2, 67) = 12.923$, $p < 0.001$], with the highest values in non-GDM group.

Body composition and anthropometrics

The measurements were made between 2nd and 7th day of the neonate's life, with mode equal to 3 days of life. The newborns in each of the study groups had similar current body weight [$F(2, 67) = 2.894$, $p > 0.05$], length [$F(2, 67) = 0.266$, $p > 0.05$], and head circumference [$F(2, 67) = 0.12$, $p > 0.05$], as well as BMI [$F(2, 67) = 1.859$, $p > 0.05$] and PI [$F(2, 67) = 2.792$, $p > 0.05$]. No significant differences were found in body water compartments: TBW [$F(2, 67) = 1.038$, $p > 0.05$], TBW% [$F(2, 67) = 1.440$, $p > 0.05$], ECW [$H(2, 70) = 2.903$, $p > 0.05$], ICW [$F(2, 67) = 1.053$, $p > 0.05$], E/I [$H(2, n = 70) = 1.077$, $p > 0.05$]; body fat: FBM [$F(2, 67) = 2.758$, $p > 0.05$], FBM% [$F(2, 67) = 1.610$, $p > 0.05$]; and fat-free mass: LBM [$F(2, 67) = 2.071$, $p > 0.05$], LBM% [$F(2, 67) = 2.174$, $p > 0.05$]. The detailed results are summarized in Table 2.

Maternal factors

To assess the impact of maternal factors on neonatal birth weight, TBW and FBM, an univariate regression (generalized linear model) was performed. The analysis considered the study group (equal to the level of disturbances in glucose metabolism), maternal age, parity, gravidity, BMI before the pregnancy, weight gain during the pregnancy, and medical history of hypothyroidism, hypertension, nicotinism. Among all factors: belonging to a particular study group and maternal history of hypothyroidism were found significant. Based on AIC values, the following factors: belonging to a particular study group and maternal history of hypothyroidism, maternal weight gain during the pregnancy were chosen as best-fitting predictors of neonatal anthropometrics and body composition. The results are presented in Supplemental Table 1.

Cluster analysis

Based on the identified three factors: belonging to a particular study group, maternal history of hypothyroidism and weight gain during pregnancy, a classification tree of patients was created (Suppl. Fig. 1). The dendrogram presents four types of patients — the characteristics of identified types of patients are presented in Table 3. 'Cluster 1' included newborns of mothers diagnosed with gestational

Table 3. Characteristics of newborns in clusters, following one-way ANOVA

Cluster	n	Study group			History of hypothyroidism			Weight gain during pregnancy [kg] (Mean ± SD)
		GDM G1 (n)	GDM G2 (n)	Non-GDM (n)	Chronic (n)	Gestational (n)	None (n)	
1	23	10	13	0	0	0	23	11.1 ± 5.7
2	27	11	16	0	18	9	0	11.2 ± 5.7
3	14	0	0	14	0	0	14	16.5 ± 5.9
4	6	0	0	6	2	4	0	15.6 ± 1.7
F statistic	-	n/a			n/a			8.31
p value	-	n/a			n/a			< 0.001

GDM — gestational diabetes; n/a — non applicable

Table 4. Multiple comparisons between clusters (post-hoc Turkey-Kramer and Dunn's test p values) for selected parameters of neonatal anthropometrics and body composition

Difference between clusters	Birth weight [kg]	TBW [l]	TBW%	ECW [l]	ICW [l]	E/I	FBM [kg]	FBM%
1–2	0.987	0.833	0.761	1.0	0.821	1.0	0.978	1.0
1–3	0.099	0.298	0.905	0.605	0.267	1.0	0.061	0.135
1–4	1.0	0.678	0.104	0.524	0.599	1.0	0.859	0.815
2–3	0.043*	0.062	0.999	1.0	0.050	1.0	0.021*	0.109
2–4	0.998	0.929	0.312	1.0	0.888	1.0	0.946	0.823
3–4	0.348	0.113	0.327	1.0	0.078	1.0	0.067	0.097

* — statistically significant (α = 0.05); TBW — total body water, ECW — extracellular water, ICW — intracellular water, E/I — extracellular/intracellular water ratio, FBM — fat mass

diabetes, without any thyroid disfunctions. 'Cluster 2' included newborns of mothers diagnosed both with gestational diabetes and hypothyroidism. 'Cluster 3' included newborns of healthy mothers, without any diabetic nor thyroid disorders. 'Cluster 4' included newborns of non-diabetic mothers with concomitant hypothyroidism. The highest maternal weight gain was observed in 'Cluster 2'. The post-hoc Turkey-Kramer test revealed differences in maternal weight gain in pregnancy between clusters, as follows: 'Cluster 1' — 'Cluster 2' p = 0.999; 'Cluster 1' — 'Cluster 3' p < 0.001; 'Cluster 1' — 'Cluster 4' p = 0.057; 'Cluster 2' — 'Cluster 3' p < 0.001; 'Cluster 2' — 'Cluster 4' p = 0.047; 'Cluster 3' — 'Cluster 4' p = 0.983. Chi-square analysis revealed a non-significant difference between clusters in maternal weight gain during pregnancy in reference to pre-gestational BMI [$\chi 2$ (6, n = 70) = 11.12, p = 0.08]. In 'Cluster 3', weight gain above recommendations was found in 10/14 mothers (71.4%), while in 'Cluster 1' — in 5/23 mothers (21.7%), in 'Cluster 2' — in 8/27 mothers (29.6%) and in 'Cluster 4' — in 3/6 mothers (50.0%). Weight gain within recommendations was achieved by 11/23 (47.8%) mothers in 'Cluster 1', 10/27 (37.0%) in 'Cluster 2', 3/14 (21.4%) in 'Cluster 3', and 2/6 (33.3%) in 'Cluster 4.' The remaining mothers in each of the clusters had weight gain below recommendations.

From the statistical comparison of clusters, which is presented in Table 4, we can see those newborns in 'Cluster 2' and 'Cluster 3' differed significantly in terms of: birth weight and FBM. Although, there were no other significant differences between the clusters of newborns, several differences in general results of anthropometric and body composition measurements can be observed. Mean (SD) newborns' birth weight in each of the clusters was: 'Cluster 1' 3.39 (± 0.49) kg, 'Cluster 2' 3.35 (± 0.51) kg, 'Cluster 3' 3.77 (± 0.41) kg, 'Cluster 4' 3.39 (± 0.22) kg. Considering total body water and body water compartments, mean (SD) values were found as follows: 'Cluster 1': TBW 2.6 (± 0.4) l, TBW% 83.4 (± 6.7)%, ECW 0.9 (± 0.2) l, ICW 1.8 (± 0.2) l, E/I 0.5 (±0.1); 'Cluster 2': TBW 2.6 (± 0.4) l, TBW% 81.6 (± 5.9)%, ECW 0.9 (± 0.2) l, ICW 1.7 (± 0.3) l, E/I 0.5 (±0.1); 'Cluster 3': TBW 2.9 (± 0.4) l, TBW% 81.9 (± 6.9)%, ECW 1.0 (± 0.2), ICW 1.9 (± 0.3), E/I 0.5 (± 0.1); 'Cluster 4': TBW 2.5 (± 0.2) l, TBW% 76.6 (± 4.4)%, ECW 0.8 (± 0.1) l, ICW 1.6 (± 0.2) l, E/I 0.5 (± 0.1). Concerning FBM and FBM%, means (SD) were: 'Cluster 1' FBM 0.26 (± 0.1) kg, FBM% 7.9 (± 2.3)%; 'Cluster 2' FBM 0.25 (± 0.1) kg, FBM% 7.9 (± 2.3)%; 'Cluster 3' FBM 0.34 (± 0.1) kg, FBM% 9.6 (± 2.0)%; 'Cluster 4' FBM 0.22 (± 0.1) kg, FBM% 7.0 (± 2.1)%. The visual comparison of results obtained in clusters is illustrated in Figure 2.

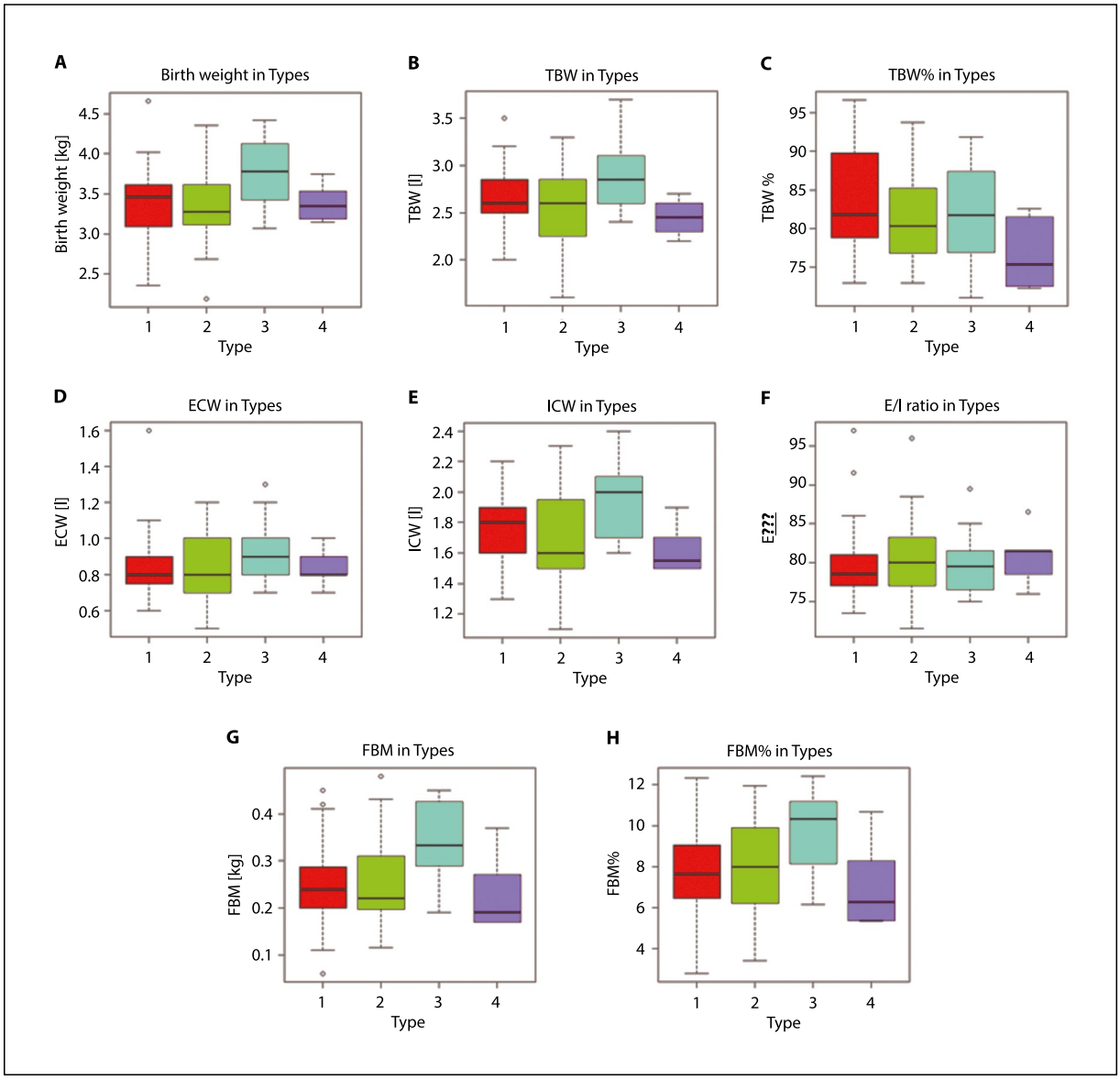


Figure 2. Plot of mean selected parameters of neonatal anthropometrics and body composition in types (clusters): **A.** Birth weight; **B.** Total body water [kg]; **C.** Total body water percentage; **D.** Extracellular water [l]; **E.** Intracellular water [l]; **F.** Extra/intracellular water ratio; **G.** Fat mass [kg]; **H.** Fat mass percentage. The horizontal axis presents numbers of clusters matching each of the box plots: 'Cluster 1' — GDM without hypothyroidism, 'Cluster 2' — GDM with hypothyroidism, 'Cluster 3' — non-GDM without hypothyroidism, 'Cluster 4' — non-GDM with hypothyroidism; notice the maternal gestational weight gain differed in clusters — details in text and in Table 3. Newborns in 'Cluster 2' and 'Cluster 3' differed significantly in terms of: birth weight and FBM

DISCUSSION

It is already well-known that severity of metabolic disorders during pregnancy and the increase in mother's weight determine the nutritional status of the newborn. Based on the literature, maternal weight gain is a significant factor that might modify influence of other maternal conditions (*e.g.*, thyroid disorders, glucose metabolic disorder, pregestational obesity or undernutrition) on fetal growth and neonatal nutritional status at birth (including body anthropometrics and fat tissue mass). Maayan-Metzger et al. [25] showed that newborns of mothers whose weight gain exceeded the recommended norms had higher birth weight and were more likely to be born by caesarean section. Moreover, these mothers were diagnosed with gestational diabetes requiring insulin therapy. Similar research results were obtained by Wang et al. [26] — among the studied women with diagnosed gestational diabetes, excessive gestational weight gain was a significant risk factor for fetal macrosomia [OR 2.884, 95% CI 1.385–6.004]. A significant effect on the development of the fetus was also demonstrated regarding high fasting blood glucose [OR 1.933, 95% CI 1.126–3.316] and elevated serum triglycerides in the third trimester of

pregnancy [OR 1.235, 95% CI 1.053–1.449]. In the study conducted by Abreu et al. [27], newborns of diabetic mothers had higher body fat content than newborns from healthy mothers. However, the main predictors of fat mass were maternal BMI before pregnancy [OR 6.75; 95% CI 2.36–11.1] and pregnancy weight gain [OR 5.64; 95% CI 1.16-10.1].

Considering hypothyroidism, Zhang et al. [28] found that persistently low levels of maternal fT3 and fT4 during the pregnancy increase a risk of large for gestational age (LGA) birth weight in a newborn, but the role of TSH level is unclear. It was also observed that adequate treatment with levothyroxine reduced a risk of fetal and neonatal macrosomia. Similar results were obtained by Turunen et al. [29] — the higher prevalence of LGA newborn was found in hypothyroid mothers than in euthyroid mothers (OR 1.14, 95% CI 1.06–1.22). Moreover, in the studied population, maternal hypothyroidism was associated with higher risk of developing gestational diabetes and LGA in newborns, but this risk was not altered by regular levothyroxine treatment.

The results of our study seem to be consistent with the abovementioned results. In general, the biggest mean values of birth weight, TBW, ICW, FBM, FBM% were found in 'Cluster 3' including newborns of non-GDM mothers without hypothyroidism, but with the highest weight gain in pregnancy and the highest rate of weight gain above recommendations in reference to pre-gestational BMI among the whole group. Whereas the lowest mean values of birth weight, TBW, TBW%, ICW, FBM, FBM% were found in 'Cluster 4' including newborns born of non-GDM mothers diagnosed with hypothyroidism, whose mean weight gain in pregnancy was lower than in 'Cluster 3' but higher than in 'Cluster 1' and 'Cluster 2'. Values of ECW and E/I were comparable between all clusters.

The mothers participating in the study had well-controlled diabetes and regularly treated hypothyroidism. Hence, the influence of glucose disorders and hypothyroidism may not be as pronounced. However, the effect of maternal weight gain during pregnancy is clearly visible — newborns of mothers with excessive weight gain in pregnancy ('Cluster 3') were found with higher birth weight and FBM than the other newborns. On the other hand, when mean maternal weight gain was higher than in other groups, but within ranges recommended for pre-gestational BMI, its effect on neonatal body composition was not prominent ('Cluster 4' vs 'Cluster 1' or 'Cluster 2'). Furthermore, mothers diagnosed with GDM had the highest mean pre-gestational BMI, but their gestational weight gain was within normal ranges, and their newborns were generally smaller than newborns of non-GDM mothers. Thus, the results of the study indicate that maternal weight gain in pregnancy has higher impact on neonatal body composition than maternal pre-gestational BMI.

Considering the diagnosis of hypothyroidism, the mean results were comparable between newborns of GDM mothers with vs without hypothyroidism ('Cluster 2' vs 'Cluster 1'), whereas among newborns of non-GDM mothers, those born out of mothers without hypothyroidism (but highest weight gain, often exceeding recommendations) had higher values of birth weight, TBW, TBW%, ICW, FBM and FBM% ('Cluster 3' vs 'Cluster 4'). Considering the diagnosis of GDM, among newborns of mothers without hypothyroidism, those in non-GDM group had higher values of birth weight, TBW, ICW, FBM, FBM% ('Cluster 3' vs 'Cluster 1') and comparable TBW%, ECW, E/I, but also in this group maternal weight gain was significantly higher (16.5 ± 5.9 kg vs 11.2 ± 5.7 kg). In the groups of newborns of mothers diagnosed with hypothyroidism, apart from TBW% and FBM% that were moderately lower in non-GDM newborns, all mean results were found comparable ('Cluster 4' vs 'Cluster 2).

In authors' opinion, the abovementioned results and similarities between groups of patients, thus limited influence of gestational diabetes and hypothyroidism on neonatal anthropometrics in the first days of life might result from appropriate maternal treatment and good compliance with medical recommendations. However, cluster abundance is the study limitation and continuation on a larger population is necessary to clarify these results.

It needs to be emphasized that the application of taxonomic analysis has enabled us to identify significant groups of patients, based on the results of a combination of several risk factors. This approach might be helpful in explicating the pathophysiology of fetal growth and neonatal outcomes in context of maternal comorbidities.

CONCLUSIONS

Neonatal anthropometrics and body composition in the first week of life are affected by a combination of maternal factors, with prominent effects of modifiable factors such as: glycemic control in gestational diabetes, sufficient supplementation of levothyroxine in hypothyroidism and gestational weight gain. Well-controlled GDM as an individual factor did not significantly affect neonatal nutritional status. Maternal weight gain during pregnancy, with reference to recommendations based on pregestational BMI, seems to be the most important determinant of neonatal birth weight, adiposity and body water distribution. Further research is needed, as newborn body composition is likely to be an important determinant of long-term health status.

Funding statement

This research was funded by Wroclaw Medical University. The research results that are presented, were completed within the scope of the topic, as recorded in the Simple sys-

tem with the number STM.A300.20.143. The APC was funded by Wroclaw Medical University, grant no. SUB.A300.21.064.

Acknowledgments

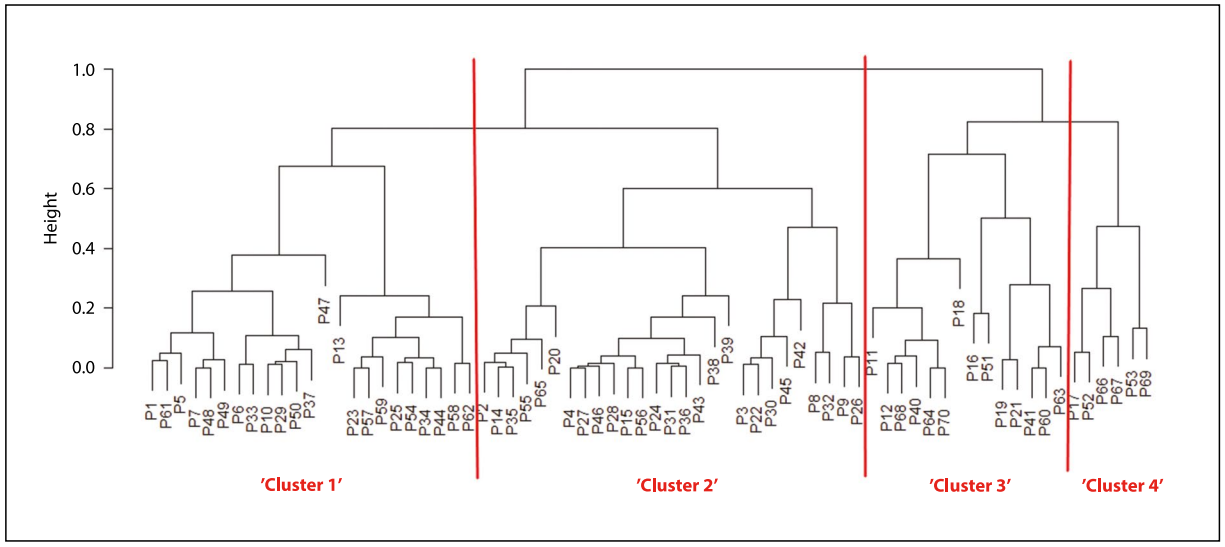
We thank Barbara and Robyn Royle for English language editing.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Schwarzenberg SJ, Georgieff MK. COMMITTEE ON NUTRITION. Advocacy for Improving Nutrition in the First 1000 Days to Support Childhood Development and Adult Health. Pediatrics. 2018; 141(2), doi: 10.1542/peds.2017-3716, indexed in Pubmed: 29358479.
2. Calkins K, Devaskar SU. Fetal origins of adult disease. Curr Probl Pediatr Adolesc Health Care. 2011; 41(6): 158–176, doi: 10.1016/j.cppeds.2011.01.001, indexed in Pubmed: 21684471.
3. Wender-Ożegowska E, Bomba-Opoń D, Brązert J, et al. Standards of Polish Society of Gynecologists and Obstetricians in management of women with diabetes. Ginekol Pol. 2018; 89(6): 341–350, doi: 10.5603/GP.a2018.0059, indexed in Pubmed: 30010185.
4. Manerkar K, Harding J, Conlon C, et al. Maternal gestational diabetes and infant feeding, nutrition and growth: a systematic review and meta-analysis. Br J Nutr. 2020; 123(11): 1201–1215, doi: 10.1017/S0007114520000264, indexed in Pubmed: 31964432.
5. Kallem VR, Pandita A, Pillai A. Infant of diabetic mother: what one needs to know? J Matern Fetal Neonatal Med. 2020; 33(3): 482–492, doi: 10.1080/14767058.2018.1494710, indexed in Pubmed: 29947269.
6. Delisle H. World Health Organization. Programming of chronic disease by impaired fetal nutrition. Evidence and implications for policy and intervention strategies. WHO/NHD/02.3, WHO/NPH/02.1. https://apps.who.int/iris/bitstream/handle/10665/67126/WHO_NHD_02.3.pdf (10.06.2021).
7. Marciniak A, Patro-Małysza J, Kimber-Trojnar Ż, et al. Fetal programming of the metabolic syndrome. Taiwan J Obstet Gynecol. 2017; 56(2): 133–138, doi: 10.1016/j.tjog.2017.01.001, indexed in Pubmed: 28420495.
8. Marken Lichtenbelt WD, Fogelholm M. Increased extracellular water compartment, relative to intracellular water compartment, after weight reduction. J Appl Physiol (1985). 1999; 87(1): 294–298, doi: 10.1152/jappl.1999.87.1.294, indexed in Pubmed: 10409587.
9. Sergi G, Lupoli L, Busetto L, et al. Changes in fluid compartments and body composition in obese women after weight loss induced by gastric banding. Ann Nutr Metab. 2003; 47(3-4): 152–157, doi: 10.1159/000070038, indexed in Pubmed: 12743467.
10. American College of Obstetricians and Gynecologists. ACOG Committee opinion no. 548: weight gain during pregnancy. Obstet Gynecol. 2013; 121(1): 210–212, doi: 10.1097/01.aog.0000425668.87506.4c, indexed in Pubmed: 23262962.
11. , et alAmerican Diabetes Association. 2. Classification and Diagnosis of Diabetes: . Diabetes Care. 2020; 43(Suppl 1): S14–S31, doi: 10.2337/dc20-S002, indexed in Pubmed: 31862745.
12. Metzger BE, Gabbe SG, Persson B, et al. International Association of Diabetes and Pregnancy Study Groups Consensus Panel. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care. 2010; 33(3): 676–682, doi: 10.2337/dc09-1848, indexed in Pubmed: 20190296.
13. Hubalewska-Dydejczyk A, Lewiński A, Milewicz A, et al. Postępowanie w chorobach tarczycy u kobiet w ciąży [Management of thyroid diseases during pregnancy]. Endokrynol Pol. 2011; 62(4): 362–81, indexed in Pubmed: 21879479.
14. Thyroid Disease in Pregnancy: ACOG Practice Bulletin, Number 223. Obstet Gynecol. 2020; 135(6): e261–e274, doi: 10.1097/AOG.0000000000003893, indexed in Pubmed: 32443080.
15. Williams B, Mancia G, Spiering W, et al. ESC Scientific Document Group. 2018 ESC/ESH Guidelines for the management of arterial hypertension. Eur Heart J. 2018; 39(33): 3021–3104, doi: 10.1093/eurheartj/ehy339, indexed in Pubmed: 30165516.
16. Marra M, Sammarco R, De Lorenzo A, et al. Assessment of Body Composition in Health and Disease Using Bioelectrical Impedance Analysis (BIA) and Dual Energy X-Ray Absorptiometry (DXA): A Critical Overview. Contrast Media Mol Imaging. 2019; 2019: 3548284, doi: 10.1155/2019/3548284, indexed in Pubmed: 31275083.
17. Lyons-Reid J, Derraik JGB, Ward LC, et al. Bioelectrical impedance analysis for assessment of body composition in infants and young children-A systematic literature review. Clin Obes. 2021; 11(3): e12441, doi: 10.1111/cob.12441, indexed in Pubmed: 33565254.
18. Mól N, Kwinta P. HOW TO DETERMINE THE NUTRITIONAL STATUS OF PRETERM BABIES?--REVIEW OF THE LITERATURE. Dev Period Med. 2015; 19(3 Pt 1): 324–329, indexed in Pubmed: 26958697.
19. Lingwood BE. Bioelectrical impedance analysis for assessment of fluid status and body composition in neonates--the good, the bad and the unknown. Eur J Clin Nutr. 2013; 67 Suppl 1: S28–S33, doi: 10.1038/ejcn.2012.162, indexed in Pubmed: 23299869.
20. Core R Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. 2020. http://www.r-project.org/ (10.06.2021).
21. Maechler M. Package 'cluster'. Cluster Analysis, extended original from Peter Rousseeuw, Anja Struyf and Mia Hubert, version 1.14.3. 2012. https://cran.r-project.org/web/packages/cluster/index.html (10.06.2021).
22. Fraley C, Raftery A, Scrucca L. Package 'mclust'. Normal mixture modeling for model-based clustering, classification, and density estimation, version 4.0. 2012. https://cran.r-project.org/web/packages/mclust/index.html (10.06.2021).
23. Marczewski E, Steinhaus H. On a certain distance of sets and the corresponding distance of functions Available online: http://matwbn.icm.edu.pl/ksiazki/cm/cm6/cm6141.pdf. Accessed: 10th June 2021. Colloq Math. 1958; 6(1): 319–327, doi: 10.4064/cm-6-1-319-327.
24. Zhai, C.X. A Note on the expectation-maximization (EM) algorithm. Chicago, IL: Department of Computer Science, University of Illinois at Urbana-Champaign. 2007. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.149.8289&rep=rep1&type=pdf (10.06.2021).
25. Maayan-Metzger A, Schushan-Eisen I, Strauss T, et al. Gestational weight gain and body mass indexes have an impact on the outcomes of diabetic mothers and infants. Acta Paediatr. 2015; 104(11): 1150–1155, doi: 10.1111/apa.13166, indexed in Pubmed: 26303990.
26. Wang Na, Ding Y, Wu J. Effects of pre-pregnancy body mass index and gestational weight gain on neonatal birth weight in women with gestational diabetes mellitus. Early Hum Dev. 2018; 124: 17–21, doi: 10.1016/j.earlhumdev.2018.07.008, indexed in Pubmed: 30081354.
27. Abreu LRS, Shirley MK, Castro NP, et al. Gestational diabetes mellitus, pre-pregnancy body mass index, and gestational weight gain as risk factors for increased fat mass in Brazilian newborns. PLoS One. 2019; 14(8): e0221971, doi: 10.1371/journal.pone.0221971, indexed in Pubmed: 31465493.
28. Zhang C, Yang Xi, Zhang Y, et al. Association Between Maternal Thyroid Hormones and Birth Weight at Early and Late Pregnancy. J Clin Endocrinol Metab. 2019; 104(12): 5853–5863, doi: 10.1210/jc.2019-00390, indexed in Pubmed: 31216012.
29. Turunen S, Vääräsmäki M, Männistö T, et al. Pregnancy and Perinatal Outcome Among Hypothyroid Mothers: A Population-Based Cohort Study. Thyroid. 2019; 29(1): 135–141, doi: 10.1089/thy.2018.0311, indexed in Pubmed: 30417761.



Supplemental figure 1. Dendrogram of the newborns. The y–axis (height) shows the value of distance metric (dissimilarity) between clusters. Horizontal bars indicate the points where clusters are merged. P identifies each of the 70 patients

Supplemental table 1. Results of univariate regression analysis

Variable		Coefficient	p value	AIC
Birth weight	Study group	–0.149	0.032*	97.38**
	Maternal age	–0.004	0.723	102.03
	Parity	0.062	0.355	101.27
	Gravidity	0.069	0.213	100.55
	Maternal BMI before the pregnancy	0.017	0.112	99.53
	Maternal weight gain during the pregnancy	0.019	0.059	98.48**
	Medical history of hypothyroidism	–0.114	0.088	99.13**
	Medical history of hypertension	0.083	0.512	100.94
	Medical history of nicotinism	–0.127	0.279	101.71
TBW	Study group	–0.073	0.211	73.42
	Maternal age	–0.011	0.315	73.99
	Parity	0.082	0.135	72.73
	Gravidity	0.075	0.101	72.26
	Maternal BMI before the pregnancy	0.009	0.286	73.86
	Maternal weight gain during the pregnancy	0.015	0.085	71.98**
	Medical history of hypothyroidism	–0.123	0.013*	69.42**
	Medical history of hypertension	0.007	0.943	75.04
	Medical history of nicotinism	0.145	0.159	72.99
FBM	Study group	0.033	0.021*	–126.33**
	Maternal age	<0.001	0.850	–120.83
	Parity	0.004	0.772	–120.88
	Gravidity	0.004	0.755	–120.89
	Maternal BMI before the pregnancy	0.002	0.374	–121.61
	Maternal weight gain during the pregnancy	0.002	0.352	–121.69
	Medical history of hypothyroidism	–0.024	0.075	–124.08**
	Medical history of hypertension	–0.026	0.286	–121.97
	Medical history of nicotinism	–0.001	0.967	–120.79

*— significant at $p < 0.05$; ** — best–fitting variables according to AIC values; TBW — total body water; FBM — fat mass; BMI — body mass index; AIC — Akaike information criterion

VIA MEDICA

ORIGINAL PAPER / OBSTETRICS

Ginekologia Polska
2023, vol. 94, no. 2, 129–134

ISSN 0017–0011, e-ISSN 2543–6767

DOI 10.5603/GP.a2022.0037

Influence of gestational diabetes in twin pregnancy on the condition of newborns and early neonatal complications

Bartlomiej Myszkowski[1], Agata Stawska[1], Malgorzata Glogiewicz[1], Marta I. Sekielska-Domanowska[1], Sawa Wisniewska-Cymbaluk[1], Rafal Adamczak[1], Jaroslaw Lach[1], Wojciech Cnota[2], Mariusz Dubiel[1]

[1]Department of Obstetrics, Gynecology and Gynecological Oncology, Faculty of Medicine, Collegium Medicum of Nicolaus Copernicus University, Bydgoszcz, Poland

[2]Clinical Department of Perinatology, Gynaecology and Obstetrics in Ruda Slaska, Medical University of Silesia, Ruda Slaska, Poland

ABSTRACT

Objectives: Diabetes mellitus is the most common metabolic complication in pregnancy and increasing worldwide. In Europe, it occurs in 3–5% of pregnant women. The rate of twin pregnancy has been increased similarly to gestational diabetes mellitus (GDM). Twin pregnancy is associated with a higher complication rate compared to singleton pregnancy. The growing prevalence of GDM and twin pregnancy has given rise to their increasing concurrent presentation.

Material and methods: The retrospective analysis included 212 twin-pregnant patients. The analysis excluded cases of miscarriage and early fetal death in the first trimester of pregnancy. The influence of GDM on the condition of newborns and mothers after delivery was analyzed. For statistical analysis R 3.6.2 software was used.

Results: No statistically significant relationship between GDM and Non-GDM group and periparturient complications was found. Birth weight was significantly higher in the GDM G2 group. Apgar Score was the lowest in the GDM G1 group. In the group of larger newborns of the GDMG1 group respiratory distress syndrome (RDS) , a higher incidence of second-degree intracranial bleeding and grade II of preterm retinopathy were observed. There was no statistically significant relationship between GDM G1, GDM G2 and other neonatal complications.

Conclusions: In summary, our results indicate that GDM in twin pregnancy does not increase the risk of cesarean section but increases some neonatal complications. In conclusion women with twin pregnancies complicated by GDM require specialist care during pregnancy and childbirth should take place in a third-level reference center.

Key words: twins; GDM; newborn; neonatal; complications

Ginekologia Polska 2023; 94, 2: 129–134

INTRODUCTION

Diabetes mellitus is the most common metabolic complication in pregnancy and increasing worldwide. In Europe, it occurs in 3–5% of pregnant women. That increase is mainly due to older maternal age, rising rate of obesity and more stringent diagnostic criteria [1, 2]. There are two types of diabetes in pregnancy: gestational diabetes mellitus (GDM) when hyperglycemia is first diagnosed in pregnancy and pregestational diabetes mellitus (PGDM) when woman with any type of diabetes is pregnant. [3, 4] In Poland gestational diabetes mellitus is diagnosed according to recommendations of the Polish Diabetes Society of 2020. The gestational diabetes mellitus is divided into diabetes treated by lifestyle modification with diet therapy (GDM G1) and diabetes treated by insulin therapy (GDM G2).

Hyperglycemia has a great influence on the development of the fetus and the condition of the newborn. Adverse perinatal outcomes include macrosomia, birth

Corresponding author:
Marta I. Sekielska-Domanowska
4 Szafirowa St, 85-790 Bydgoszcz, Poland
e-mail: marta.sekielska@gmail.com

Received: 22.02.2022 Accepted: 1.04.2022 Early publication date: 26.05.2022

injury, greater rate of surgical deliveries, risk of neonatal severe respiratory distress syndrome and neonatal metabolic disorders such as hypoglycemia, hyperbilirubinemia, hypocalcemia [3, 5, 6].

The rate of twin pregnancy has been increased similarly to GDM. It is mainly caused by greater use of assisted reproductive technology and shift toward an older maternal age [2].Unfortunately twin pregnancy is also associated with a higher complication rate compared to singleton pregnancy. Women with twin pregnancy are exposed to miscarriage, gestational diabetes mellitus (GDM), hypertension, anemia, placenta previa, placental abruption, preterm labour, premature rupture of membranes [2, 7, 8]. The growing prevalence of GDM and twin pregnancy has given rise to their increasing concurrent presentation [3].

Some studies have shown an increased risk for gestational hypertension, preeclampsia, cesarean section in women with twin pregnancy and GDM compared to women with twin pregnancy without GDM [8]. The aim of our study is to assess whether the increased risk associated with twin pregnancy is exacerbated in the presence of GDM. One of the most frequent complication of newborns is respiratory distress syndrome (RDS) which remains a significant health problem. Does gestational diabetes mellitus significantly increase the risk of its occurrence? In addition, we want to show whether the type of GDM (GDM G1 or GDM G2) has an influence on the type of pregnancy complications and condition of the newborns.

MATERIAL AND METHODS

A retrospective analysis of computer documentation was performed on patients with multiple twin pregnancies, who gave birth in the Department of Maternal and Fetal Medicine, Gynecology and Neonatology in Bydgoszcz over the period 2014–2018, as well as of computer neonatal documentation of newborns — a total of 212 patients.

The study was based on the diagnosis from the Hospital Treatment Information Card and diabetes consultations during hospitalization.

In order to identify fetuses based on medical records and to facilitate statistical calculations, a division into a larger and smaller newborn was introduced in the analyses of the condition of the newborn, neonatal complications and congenital abnormalities.

In the analysis cases of miscarriage, early fetal death in the first trimester of pregnancy and patients who were treated during pregnancy in the Department of Maternal and Fetal Medicine, Gynecology and Neonatology but delivered in other units were excluded.

The analysis of quantitative variables (*i.e.*, expressed in number) was performed by calculating the mean, standard deviation, median, quartiles, minimum and maximum.

The analysis of qualitative (*i.e.*, non-numeric) variables was performed by calculating the number and percentage of occurrences of each value.

The comparison of the values of qualitative variables in the groups was performed using the chi-square test (with Yates's correction for 2×2 tables) or the Fisher's exact test where low expected frequencies appeared in the tables.

The comparison of the values of quantitative variables in two groups was performed using the Mann-Whitney test.

The comparison of the values of quantitative variables in three or more groups was performed using the Kruskal-Wallis test. After detecting statistically significant differences, post-hoc analysis with Dunn's test was performed to identify the statistically significantly different groups.

The comparison of the values of quantitative variables in two repeated measurements was performed with the Wilcoxon test for bonded pairs.

A significance level of 0.05 was adopted in the analysis, so all p values below 0.05 were interpreted as showing significant relationships.

RESULTS

In twin pregnancies complicated by gestational diabetes, increased pregnancy supervision is obligatory, both on the part of obstetrics and diabetes. The distribution of twin pregnancies in terms of the time of labour is similar in the group with gestational diabetes and in the group without diabetes.

In the group with gestational diabetes there was no case of preterm labour before the 28th week of pregnancy. Four pregnancies were finished before 32 weeks of gestation, but no statistically significant correlation was found between GDM and delivery before 32 weeks of gestation.

Vast majority of patient, both with and without GDM, gave birth in the period 33–37 t.c. In both groups, more than one third of patients gave birth after 37 weeks of pregnancy. There was no statistically significant relationship between GDM and intrauterine death. There was no association between GDM in twin pregnancies and the type of delivery. In both cases, caesarean section was by far the most popular choice (Tab. 1).

There was no statistically significant relationship between GDM and complications of early puerperium such as: puerperal anemia, blood loss requiring blood transfusion, perinatal hysterectomy, pre-eclampsia, eclampsia, post-dural syndrome, endometritis, postpartum depression.

Birth weight was significantly higher in the group of patients treated with insulin than in the group without insulin in the case of the smaller children of the couple (Fig. 1). In larger children, the birth weight was significantly higher in the group with insulin than in the group treated with diet, where it was significantly higher than in the

Table 1. The gestational age at the end of pregnancy and the type of delivery depending on the occurrence of gestational diabetes mellitus (GDM)

Parameter		GDM		p
		No (n = 177)	Yes (n = 33)	
Gestational age at birth	Extremely premature delivery	6 (3.4%)	0 (0.0%)	p = 0.489
	Very premature delivery	11 (6.2%)	4 (12.1%)	
	Moderately premature delivery	96 (54.2%)	16 (48.5%)	
	Delivery at the right time	64 (36.2%)	13 (39.4%)	
Type of delivery	Natural delivery	22 (12.4%)	1 (3.0%)	p = 0.138
	Caesarean section	155 (87.6%)	32 (97.0%)	

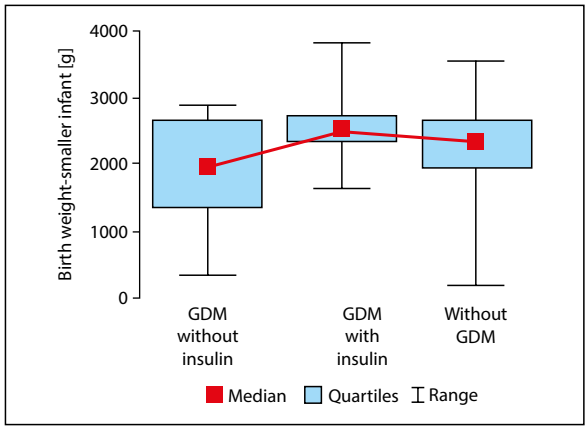


Figure 1. Weight of the smaller infant from a twin pregnancy depending on the onset and type of gestational diabetes mellitus (GDM)

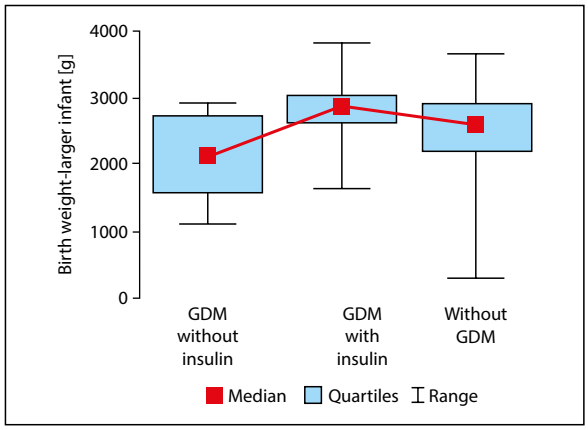


Figure 2. Weight of the larger infant from a twin pregnancy depending on the onset and type of gestational diabetes mellitus (GDM)

group without GDM (Fig. 2). The birth weight of smaller newborns was on average: 1,847.8 g (median 1930 g) in the GDM G1 group, 2513.3 g (median 2475 g) in the GDM

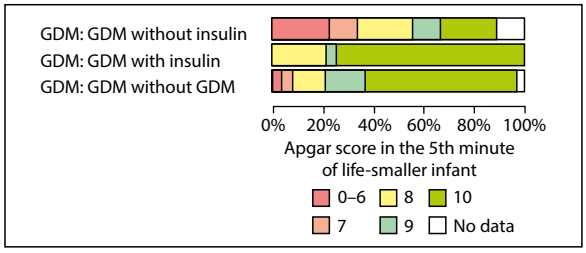


Figure 3. Apgar score in the 5th minute of life of the smaller infant from a twin pregnancy depending on the onset and type of gestational diabetes mellitus (GDM)

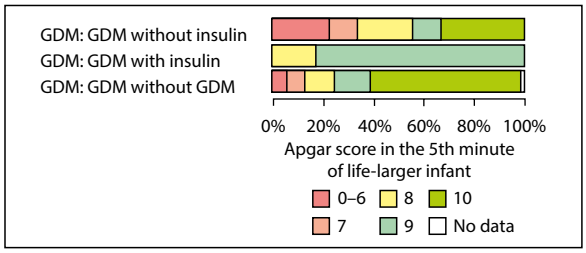


Figure 4. Apgar score in the 5th minute of life of the larger infant from a twin pregnancy depending on the onset and type of gestational diabetes mellitus (GDM)

G2 group and 2236.0 g (median 2320 g) in the group without GDM. The birth weight of larger newborns was on average: 2,085.6 g (median 2,130 g) in the GDM G1 group, 2,791.3 g (median 2,860 g) in the GDM G2 group and 2502.2 g (median 2,640 g) in the group without GDM.

The Apgar score in the 5th minute was the best in the group without GDM, slightly worse in the group with insulin, and the worst in the group without insulin in both smaller and larger children. In addition, larger children with GDM G1 pregnancies presented lower Apgar scores in the 1st minute (Fig. 3 and 4).

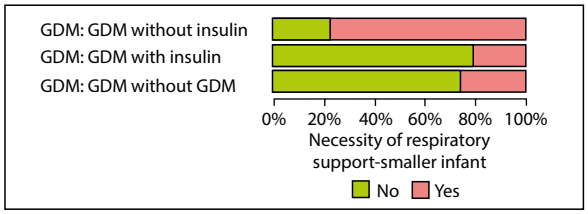


Figure 5. Necessity of respiratory support in the smaller infant from a twin pregnancy depending on the onset and type of gestational diabetes mellitus (GDM)

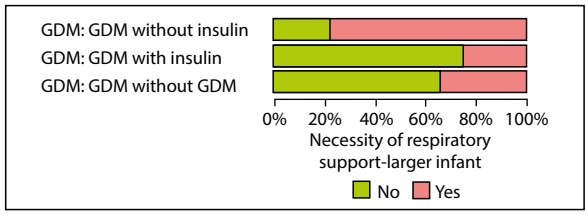


Figure 6. Necessity of respiratory support in the larger infant from a twin pregnancy depending on the onset and type of gestational diabetes mellitus (GDM)

The need for respiratory support was the rarest in the GDM G2 group: 20.8% in the smaller children and 25% in the larger children, slightly more frequent in the group without GDM: 26% in the smaller children and 33.9% in the larger children, and the most frequent in the GDM group G1: 77.8% in both smaller and larger children (Fig. 5 and 6).

In the group of larger newborns, the average duration of stay in the Neonatology Department turned out to be significantly longer in the case of GDM G1 compared to the GDM G2 group and the non-diabetic group and amounted to 26,1 days (median 20), 9 days (median 5) and 11,4 days (median 7) respectively. In smaller infants, a similar relationship was not confirmed ($p > 0.05$ in the Kruskal-Wallis test) (Fig. 7 and 8).

There was no statistically significant relationship between GDM G1, GDM G2, and birth body length, discharge weight, and NICU admission in the case of the smaller child of the twin pair.

In the group of smaller infants of twin pregnancies complicated by GDM G1, anemia was more frequent (Tab. 2). Statistical significance was not demonstrated in the group of larger infants.

In the group of larger newborns from twin pregnancies complicated by GDM G1, respiratory distress syndrome (RDS) was diagnosed more often. In the group of smaller children, there is no significant relationship between GDM G1 and RDS. However, it is necessary to consider the more frequent incidence of more serious degrees of RDS requiring mechanical support in both groups of newborns with GDM G1.

Grade II preterm retinopathy occurred more frequently in the group of larger infants with GDM G1, but with no significant association in the group of smaller infants.

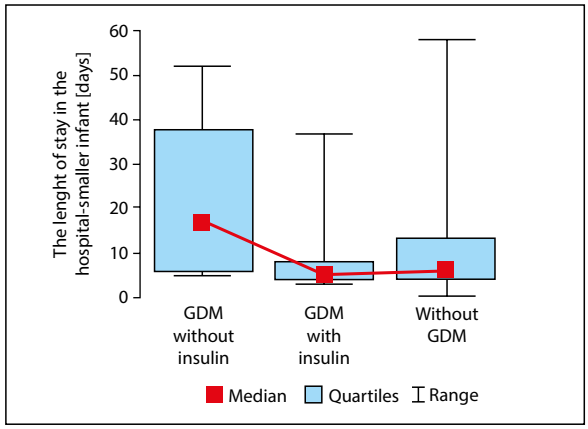


Figure 7. The length of stay in the hospital of the smaller infant from a twin pregnancy depending on the onset and type of gestational diabetes mellitus (GDM)

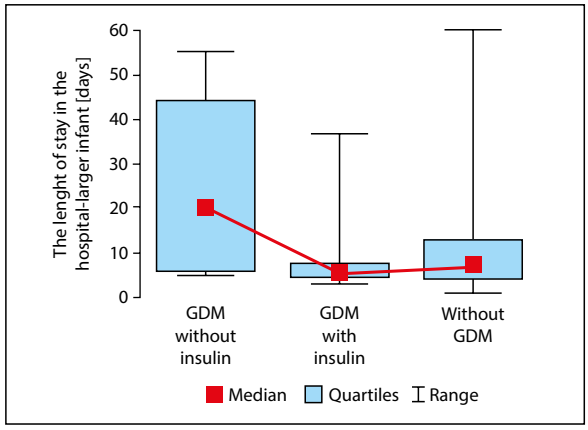


Figure 8. The length of stay in the hospital of the larger infant from a twin pregnancy depending on the onset and type of gestational diabetes mellitus (GDM)

Table 2. The incidence of anemia of the smaller newborn from a twin pregnancy depending on the onset and type of gestational diabetes mellitus (GDM)

Complications – smaller infant	GDM			p
	GDM G1 (n = 9)	GDM G2 (n = 24)	No GDM (n = 177)	
Anemia	3 (33.3%)	1 (4.2%)	14 (7.9%)	p = 0.044

Table 3. The incidence of complications in the larger infant: II-degree intracranial bleeding, respiratory distress syndrome, II-degree premature retinopathy depending on the onset and type of gestational diabetes mellitus (GDM)

Complications – larger infant	GDM			p
	GDM G1 (n = 9)	GDM G2 (n = 24)	No GDM (n = 177)	
II-degree intracranial bleeding	2 (22.2%)	0 (0.0%)	4 (2.3%)	p = 0.027
respiratory distress syndrome	6 (66.7%)	5 (20.8%)	57 (32.2%)	p = 0.041
II-degree premature retinopathy	2 (22.2%)	0 (0.0%)	2 (1.1%)	p = 0.02

Gestational diabetes G1 and G2 are not associated with grade I premature retinopathy.

In the group of larger children with GDM G1 pregnancies, a higher incidence of second-degree intracranial bleeding was observed, but with no apparent correlation in smaller infants. There was no relationship between more severe degrees of IVH and GDM G1, GDM G2, both in the group of smaller and larger newborns (Tab. 3).

There was no statistically significant relationship between GDM G1, GDM G2 and other neonatal complications, such as: polycythemia, neonatal haemorrhagic disease, neonatal hypotrophy, persistent fetal circulation, NEC, pneumonia, 1st degree preterm retinopathy, perinatal hypoxia, death of a live born newborn , jaundice.

There was no statistically significant relationship between GDM G1, GDM G2 and birth defects. However, it should be considered that patients with diagnosed hemodynamically significant fetal heart defects were transferred to highly specialized units with the option of cardiac surgery.

DISCUSSION

Gestational diabetes is a condition of impaired glucose tolerance that was first diagnosed during pregnancy. In Poland, all pregnant women are covered by diabetes prevention in line with the Recommendations of the Polish Society of Gynecologists and Obstetricians. The panel of tests in the first trimester of pregnancy includes fasting glycaemia, among others. In case of abnormalities, further diagnosis is implemented depending on the fasting glycaemia.

Additionally, an oral glucose loading test is performed at 24–28 weeks of gestation. If gestational diabetes is diagnosed, the patient is provided with diabetes care, and childbirth should take place in a third-level reference center [9].

Studies on the impact of diabetes in pregnancy on the fetus have shown that maternal diabetes is significantly related to neonatal RDS, even if the gestational age at delivery exceeded 34 + 0 [5, 6].

Perhaps the more frequent preterm infant retinopathy in newborns of mothers with GDM results from more frequent respiratory support as the studies of extreme premature infants show that significant risk factors for retinopathy are mechanical ventilation, low gestational age, low birth weight, and blood transfusion [10, 11].

Sheehan A. et al. [7] compared the course of single and twin pregnancies with and without GDM. It has been shown that twin pregnancy, compared to the effects of GDM, is the most important risk factor for an adverse perinatological outcome, including very premature delivery, cesarean section and neonatal complications. GDM in twin pregnancies did not increase the risk of neonatal complications, except for hypoglycemia. The study of Guillen-Sacoto et al. [12] has shown the higher risk for the newborns of severe SGA, hypoglycemia, and polycythemia in twin pregnancies.

The meta-analysis carried out by McGrath et al. [1] did not show a relationship between gestational diabetes in twin pregnancy and respiratory complications, hypotrophy, or disturbed carbohydrate metabolism in the newborn. Nevertheless, newborns from a twin pregnancy complicated with GDM showed a slightly lower birth weight and more frequent admission to the ICU.

Worse birth status in newborns with GDM G1 twin pregnancies compared to GDM G2 and in patients without glucose tolerance impairment may result from insufficient compliance with dietary recommendations and, consequently, lack of glycemic control. Insulin therapy in pregnancy is associated with greater discipline of pregnant patients in relation to a diet with limited easily digestible carbohydrates. There is a known relationship between mechanical ventilation of the newborn and preterm retinopathy requiring laser therapy [13]. Moderate and late prematurity and its complications may be related to inadequate glycemic control and dietary adherence in pregnant women. Observational studies by Antoniou et al. showed a relationship between the increased value of HBA1c at the end of pregnancy and prematurity [14].

CONCLUSIONS

In summary, our results indicate that GDM in twin pregnancy does not increase the risk of cesarean section. Caesarean section was the most popular choice in both groups. A higher percentage of newborns were born in a severe or moderate condition in the group of twin pregnancies complicated by GDM. Newborns from twin pregnancies complicated by GDM G1 presented respiratory distress syndrome (RDS) more often. In conclusion, women with twin pregnancies complicated by GDM require specialist care during pregnancy and childbirth should take place in a third-level reference center.

Conflict of interest

All authors declare no potential conflicts of interest.

REFERENCES

1. McGrath RT, Hocking SL, Scott ES, et al. Outcomes of twin pregnancies complicated by gestational diabetes: a meta-analysis of observational studies. J Perinatol. 2017; 37(4): 360–368, doi: 10.1038/jp.2016.254, indexed in Pubmed: 28079866.
2. Kim Y, Hong SY, Kim SY, et al. Obstetric and neonatal outcomes of gestational diabetes mellitus in twin pregnancies according to changes in its diagnostic criteria from National Diabetes Data Group criteria to Carpenter and Coustan criteria: a retrospective cohort study. BMC Pregnancy Childbirth. 2022; 22(1): 9, doi: 10.1186/s12884-021-04361-9, indexed in Pubmed: 34979991.
3. Wender-Ożegowska E, Bomba-Opoń D, Brązert J. Standardy Polskiego Towarzystwa Ginekologów i Położników postępowania u kobiet z cukrzycą. Ginekologia i Perinatologia Praktyczna. 2017; 2(5): 215–229.
4. Polskie Towarzystwo Diabetologiczne. Zalecenia kliniczne dotyczące postępowania u chorych na cukrzycę 2020. Diabetologia praktyczna. 2020; 6(1): 1–106.
5. Mortier I, Blanc J, Tosello B, et al. Is gestational diabetes an independent risk factor of neonatal severe respiratory distress syndrome after 34 weeks of gestation? A prospective study. Arch Gynecol Obstet. 2017; 296(6): 1071–1077, doi: 10.1007/s00404-017-4505-7, indexed in Pubmed: 28948345.
6. Li Y, Wang W, Zhang D. Maternal diabetes mellitus and risk of neonatal respiratory distress syndrome: a meta-analysis. Acta Diabetologica. 2019; 56(7): 729–740.
7. Sheehan ACM, Umstad MP, Cole S, et al. Does Gestational Diabetes Cause Additional Risk in Twin Pregnancy? Twin Res Hum Genet. 2019; 22(1): 62–69, doi: 10.1017/thg.2018.72, indexed in Pubmed: 30661509.
8. Hung TH, Hsieh TT, Shaw SW, et al. Risk factors and adverse maternal and perinatal outcomes for women with dichorionic twin pregnancies complicated by gestational diabetes mellitus: A retrospective cross-sectional study. J Diabetes Investig. 2021; 12(6): 1083–1091, doi: 10.1111/jdi.13441, indexed in Pubmed: 33064935.
9. Standardy Polskiego Towarzystwa Ginekologicznego postepowania u kobiet z cukrzyca--aktualizacja [Actualisation of Polish Gyneacological Society standards of medical care in management of women with diabetes]. Ginekologia Polska. 2014; 85(6): 476–478.
10. Tomé VA, Vieira JF, Oliveira LB. Estudo da retinopatia da prematuridade em um hospital universitário [Study of retinopathy of prematurity in a university hospital]. Arquivos Brasilerios de Oftalmologia. 2011; 74(4): 279–282.
11. Figueras-Aloy J, Alvarez-Domínguez E, Morales-Ballus M, et al. [Early administration of erythropoietin in the extreme premature, a risk factor for retinopathy of prematurity?]. An Pediatr (Barc). 2010; 73(6): 327–333, doi: 10.1016/j.anpedi.2010.09.001, indexed in Pubmed: 20951656.
12. Guillén-Sacoto MA, Barquiel B, Hillman N, et al. Gestational diabetes mellitus: glycemic control during pregnancy and neonatal outcomes of twin and singleton pregnancies. Endocrinol Diabetes Nutr (Engl Ed). 2018; 65(6): 319–327, doi: 10.1016/j.endinu.2018.01.011, indexed in Pubmed: 29685731.
13. Choi YB, Lee J, Park J, et al. Impact of Prolonged Mechanical Ventilation in Very Low Birth Weight Infants: Results From a National Cohort Study. J Pediatr. 2018; 194: 34–39.e3, doi: 10.1016/j.jpeds.2017.10.042, indexed in Pubmed: 29198532.
14. Antoniou MC, Gilbert L, Gross J, et al. Potentially modifiable predictors of adverse neonatal and maternal outcomes in pregnancies with gestational diabetes mellitus: can they help for future risk stratification and risk-adapted patient care? BMC Pregnancy Childbirth. 2019; 19(1): 469, doi: 10.1186/s12884-019-2610-2, indexed in Pubmed: 31801465.

VIA MEDICA

ORIGINAL PAPER / OBSTETRICS

Ginekologia Polska
2023, vol. 94, no. 2, 135–140

ISSN 0017–0011, e-ISSN 2543–6767

DOI 10.5603/GP.a2022.0141

The nightmare of obstetricians — the placenta accreta spectrum in primiparous pregnant women

Ülkü Ayşe Türker Aras, Engin Korkmazer, Emin Üstünyurt

Departmant Of Obstetrics And Gynecology, Bursa High Specialization Training And Research Hospital, Bursa, Turkey

ABSTRACT

Objectives: The incidence of PAS is increasing day by day as a life-threatening condition. The purpose of the present study was to determine the factors affecting PAS formation in primiparous pregnant women and to define possible risk factors for the mother and the baby.

Material and methods: Bursa Yüksek İhtisas Training and Research Hospital, department of obstetrics and gynecology, Bursa, Turkey, between June 2016 and December 2020. A total of 58,895 patients were included in the study. After the exclusion criteria, the study was continued with 27 primiparous PAS and 54 non-primiparous PAS patients. The primary purpose is to evaluate PAS risk factors. The secondary aim is to examine maternal and neonatal characteristics.

Results: When the parameters that are significant in terms of PAS risk factors were analyzed by Logistic Regression Analysis, it was found that the increase in age also increased the development of PAS 1.552 times (95% CI: 1.236–1.948) and a history of abortion was 7.928. times (95% CI: 1.408–44.654) and 11,007 times (95% CI: 2.059–58.832) with history of myomectomy postoperative HB values ($p < 0.001$), an estimated amount of bleeding ($p < 0.001$), need for transfusion ($p = 0.002$), and use of drains (< 0.001) were statistically significant different between two groups. When the neonatal results between patients with and without PAS were examined, birth weight ($p < 0.001$) and gestational week (< 0.001) were statistically significant.

Conclusions: PAS does not occur only in multiparous patients who have a history of previous cesarean section. It may also occur in primiparous patients and is a life-threatening condition.

Key words: spectrum of placenta accreta, high risk pregnancy, primiparous pregnancy

Ginekologia Polska 2023; 94, 2: 135–140

INTRODUCTION

Placenta accreta spectrum (PAS) is defined as the abnormal invasion of the placental tissues into the myometrium [1–3]. The diagnosis of PAS is suspected with ultrasonography and confirmed with pathological diagnosis after surgery [4]. PAS is a maternal life-threatening condition that associated with maternal mortality and morbidity [1–3]. Severe postpartum hemorrhage, need for blood transfusion, Disseminated Intravascular Coagulation (DIC), organ injury, ileus, infection, thromboembolic complications, need for intensive care, renal failure, and increased mortality and morbidity detected in cases with PAS are higher than uncomplicated pregnancies [5–7].

It is stated in the literature that the most important factor for the development of PAS is history of cesarean section before [8, 9]. The incidence of PAS is known to be 2–4.84 per 1000 birth and increasing with cesarean delivery rates throughout the globe [10, 11]. However, when the literature data were reviewed, it was seen that 38% of the patients with PAS were primiparous women [12, 13]. The purpose of the present study was to determine the factors affecting PAS formation in primiparous pregnant women and to define possible risk factors for the mother and the baby.

MATERIAL AND METHODS

The place where the study was conducted

The third-largest education and research hospital in the South Marmara region, where approximately 13.000 births are performed on an annual scale.

Corresponding author:
Ülkü Ayşe Türker Aras
Departmant Of Obstetrics And Gynecology, Bursa High Specialization Training And Research Hospital, 36100 Bursa, Turkey
e-mail: ulku1ayse@gmail.com

Received: 7.05.2022 Accepted: 13.10.2022 Early publication date: 15.12.2022

Case group

A total of 27 primiparous pregnant women who were diagnosed with PAS between June 2016 and December 2020.

Control group

A total of 54 women (1 case vs 2 controls) who were selected randomLy from among the primiparous women who delivered throughthe elective cesarean section between the same years constituted the Control Group.

Study population

The study was initiated with 58,895 patients. Multiparous patients were excluded from the study. A total of 13.105 of 21.782 primiparous women delivered through cesarean section (Fig. 1). Among these, patients with twin pregnancies were excluded from the study. A total of 29 of these patients were diagnosed with PAS Ultrasonographically in the antenatal period. When the postoperative pathology results were evaluated, 27 patients had the diagnosis of PAS. The pathology result of all of these patients was *placenta acreata*, which is the sub-parameter of PAS. The Control Group patients were selected randomLy as 1 case vs 2 controls among the pregnant women who were scheduled to have an elective primiparous cesarean section on the day of surgery of the patients who were diagnosed with PAS. In each of the PAS cases in the study, the placenta completely covers the cervical os. However, the placement of the placenta is anterior or posterior. Similarly, those with anterior or posterior placental location in the non-PAS group were included in the study by random selection. Non-Pass cases where the placenta was fundal located were not included.

Variables: The parameters that were examined in the study are given in the Table 1 of variables and their definitions

Purpose of the study

As the primary outcome: The purpose was to evaluate PAS risk factors.

As a secondary outcome:The purpose was to evaluate the estimated amount of bleeding, need for transfusion, length of hospital stay, organ injury, and wound site infection as neonatal outcomes.

Statistical analysis

The SPSS 21.0 was used for all statistical analyses (Statistical Package for the Social Sciences, Chicago, IL). A p-value of ≤ 0.05 was considered statistically significant. The Shapiro-Wilk test was used to evaluate whether or not the mean values fit the normal distribution. The *t*-test was used for the mean values with normal distribution and the Mann-Whitney U test for those who did not. The Chi-Square test was used for pairwise comparisons. The BackwardLogistic Regression test was usedfor the parameters that were significant among the risk factors.

RESULTS

The study was conducted by examining the data of 58.895 patients between June 2016 and December 2020

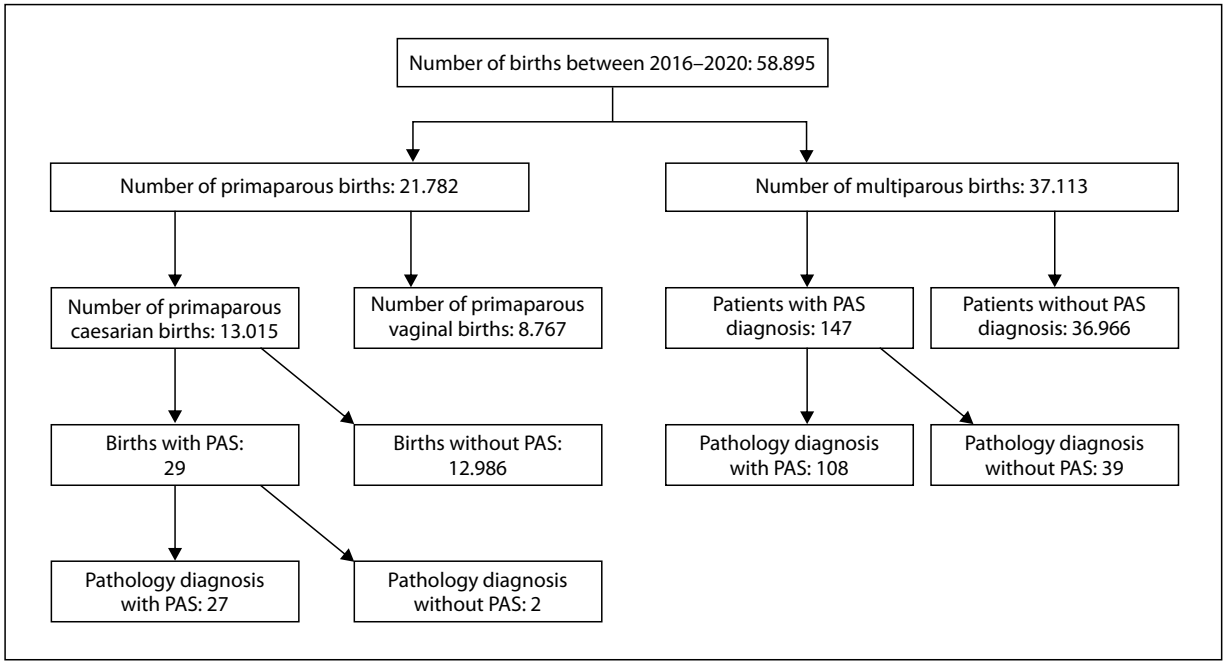


Figure 1. The patients who were includedin the study; PAS — placenta accreta spectrum

Table 1. Variables and their definitions [4]

Variables	Definition
PAS diagnosis	The diagnosis was made by experienced obstetricians and gynecologists based on transvaginal and transabdominal ultrasonography findings. Also, the diagnosis was made sure with the pathology materialsthat were taken during the surgery. Diagnostic criteria of PAS in ultrasonography were; decreased hypoechogenicity in the retroplacental area, irregular vascular areas in the placenta, increased vascularity in the myometrium layer between the uterus and bladder [4]
Risk factors for PAS	Age [years] (mean)
	BMI [kg/m^2] (mean)
	Curettagehistory (Yes/No)
	Myomectomyhistory (Yes/No)
	Assisted Reproductive Technique(Yes/No)
Intraoperative and postoperative characteristics	Hb: Hemoglobin (preoperative (one day before the operation)and postoperative 6th-hour HB values were recorded)
	Operation type: **Local Resection:** Elective cesarean section was planned for patients with PAS between 34 and 37 weeks according to the degree of invasion. The abdominal cavity was entered with the midline incision. After the peritoneal cavity was entered, exploration was performed to determine the placental invasion margins. The baby was removed from fundal incision, the umbilical cord was tied, and the placenta was left in place. The fundal incision was sutured. After that we ligated internal iliac artery and utero-ovarian ligaman with 1–0 vicryl suture for reducing bloood flow to uterus. we disect the bladder from uterus with advanced bipolar energy source. We excised the placental invasion area with 1 cm invasion free safe margin. After that placenta removed from uterus. Resection was performed with scissors and cautery, and bleeding areas were controlled. One single layer of continuous suture was used to close the transverse incision in the anterior uterine wall. We insertid bakri postpartum balloon and insuflated with 250 mL saline. We removed balloon after 24 hours of procedure. **Cesarean section:** Primiparous pregnant women who did not have PAS but were planned for elective cesarean section were included in the present study. The cavity was entered with the Pfannenstiel incision. After the peritoneal cavity was entered, exploration was performed to determine the invasion margins. After the disection of bladder, lower uterine segment was incised with a transverse incision, the baby was removed, the umbilical cord was tied, and the placenta was removed. The incision was sutured. One single layer of continuous suture was used to close the uterine incision. We insertid bakri postpartum balloon and insuflated with 250 mL saline. We removed balloon after 24 hours of procedure. **For all patients:** After the fetus and placenta were removed, 20 IU intravenous oxytocin infusion was administered, and 15 IU intravenous oxytocin infusion was continued in the first 24 hours. All patients were administered prophylactic 2-gram intravenous Cefazolin half an hour preoperatively. These patients were mobilized at the 6th hour and thromboprophylaxis was administered to the patients.
	The estimated amount of bleeding: It is defined as the bleeding from the beginning of the skin incision to the end of the labor. The amount of bleeding was calculated by taking the sum of the amount of blood that was absorbed by the gauze and the blood in the aspirator chamber.
	Need for transfusion: The obstetrician and anesthesiologist made the decision in this respect. The factors that affected blood transfusion were; preoperative anemia, amount of bleeding during surgery, and hemogram values during surgery.
	Duration of Hospitalization: Given as day(s)
	Wound siteinfection:Yes/No
Neonatal characteristics	Gestational week: Week
	Birth weight: Grams
	APGAR1-5:1 and 5th-minute APGAR Score
	NICU need: Yes/ No

PAS — placenta accreta spectrum; BMI — body mass index; NICU — Neonatal Intensive Care Unit

in the 3rd Stage Training and Research Hospital, where approximately 13.000 deliveries are recordedon an annual scale. After the exclusion criteria were applied, the study was continued with 27 PAS and 54 Non-PAS primiparous women. PAS rate was 0.2% and the incidence of primiparous PAS was 0.5% in the Study Group.

When PAS risk factors were examined, none of the patients became pregnant with the use of any assisted reproductive technique. None of them had a history of ectopic pregnancy or molar pregnancy. It was the first birth for all patients. Although no statistically significant differenceswere detected between the Body Mass Indicesof both groups (p = 0.740), statistically significant differenceswere found in terms of the history of previous abortion (p < 0.001), myomectomy history (< 0.001), and the mean age of the mothers (< 0.001) (Tab. 2).

When the parameters that were significant regarding the PAS risk factors were analyzed with the logistic regression analysis, the increase in age increased PAS development 1.552-fold (95% CI: 1.236–1.948), a history of curettage 7.928-fold (95% CI: 1.408–44.654), and history of myomectomy 11.007-fold (95% CI: 2.059–58.832) (Tab. 3).

All patients with PAS underwent local resection and Cesarean Section was performed for all cases without PAS.

Although no statistically significant differences were detected between preoperative HB values (p = 0.104), wound infection development (p = 0.895), and hospitalization durations (p = 0.463) between the patients with and without PAS, statistically significant differences were found in the postoperative HB values (p < 0.001), estimated bleeding amount (p < 0.001), the need for transfusion (p = 0.002), and the use of drains (< 0.001). Also, bladder or bowel damage wasnot detected in any patient (Tab. 4).

When the neonatal outcomes of the patients with and without PAS were examined,although 1st-minute APGAR score (p = 0.532), 5th-minute APGAR score (p = 0.70) values, and NICU need (p = 0.204) were statistically insignificant, birth weight (p < 0.001) and gestational week (< 0.001) were found to be statistically significant (Tab. 5).

Table 2. The distribution of the variables according to case-control groups

Variables Number (%)		Control (n = 54)	Case (n = 27)	P
		Number (%)		
Curettage history	2 +	10 (18.5)	17 (63.0)	< 0,001*
	1	44 (81,5)	10 (37.0)	
Myomectomy	No	47 (87.0)	10 (37.0)	< 0,001*
	Yes	7 (13.0)	17 (63.0)	
BMI	Mean ± (SD)	25.62 ± 2.79	25.85 ± 3.32	0.740**
Age	Mean ± (SD)	22.85 ± 3.92	30.52 ± 4.57	< 0.001***

*Chi-Square Test, **t-test, ***Mann-Whitney U test; BMI — body mass index; SD — standard deviation

Table 3. Placenta accreta spectrum risk factors logistic regression analyses results

Independent variables	Odds ratio	95% CI (Min–Max value)
Age	1.552	1.236–1.948
Curettage	7.928	1.408–44.654
Myomectomy	11.007	2.059–58.832

CI — confidence interval

DISCUSSION

Consistent with the literature data, the PAS rate was found to be 0.2% in the Study Group, and the incidence of primiparous PAS was 00.5%. When the literature data were reviewed, it was found that the worldwide PAS rate was reported to be 0.01–4.84%, the primiparous PAS rate was approximately 1 in 3 of all PAS cases, and in another study, this rate was reported as 2.4 per 1000 among all pregnancies [10–14]. When the previous publications on the subject were examined and in our study, although it is reported that the history of previous cesarean section is the most important factor in the development of PAS, it is seen that it can also occur in women who have not given birth before [13].

A total of 810 women die every day in the world because of complications related to childbirth [15]. PAS is among the most important causes of maternal mortality and morbidity in primiparous patients and should be examined in detail. However, when the literature data were reviewed, it is not specified in which primiparous pregnant women the clinician should be especially alert. When the results of the present study are examined, the history of myomectomy, previous abortion, and increased maternal age were found to be risk factors.

In the meta-analysis that was conducted by Iacovelli et al. [16], although increasing maternal age and myomectomy history were found to be effective in terms of PAS development in line with the current study, uterine curettage history was found to be insignificant in terms of PAS development. However, we think that this was because both multiparous and primiparous women were examined in the meta-analysis, but only the data of primiparous women were examined in the current study [16]. However, in studies that included fewer cases, it was reported that a history of curettage is a risk factor for the development of PAS as it caused endometrial damage. However, when the data of these studies were reviewed, it was found that they included both multiparous and primiparous patients [13, 17–20]. Increasing maternal age was identified as an independent risk factor in a previous study that was conducted to determine the incidence of PAS in primiparous women [14]. Also,

Table 4. Evaluation of the preoperative and postoperative characteristics of the patients

Variables		Control (n = 54) Number (%)	Case (n = 27) Number (%)	P
Preoperative HB	Mean ± SD	11.2985 ± 1.019	10.829 ± 1.083	0.104**
Postop HB	Mean ± SD	11.2204 ± 0.820	9.451 ± 1.403	< 0.001**
Estimated Bleeding	Mean ± SD	302.037 ± 191.512	523.333 ± 279.642	< 0.001**
Hospitalization	Mean ± SD	3.259 ± 0.442	3.777 ± 2.189	0.463**
Tx necessary	Yes	1 (1.9)	7 (25.9)	0.002*
	No	53 (98.1)	20 (74.1)	
Drain	Yes	5 (9.3)	24 (88.9)	< 0.001*
	No	49 (90.7)	3 (11.1)	
Type surgery	Cs	54 (100.0)	17(63.0)	< 0.001*
	Local resection	0 (0.0)	10 (37.0)	
Surgery side infection	Yes	6 (11.1)	2 (7.4)	0.895*
	No	48 (88.9)	25 (92.6)	

*Chi-Square Test; **Mann-Whitney U; SD — standard deviation; HB — Hemoglobin; Tx — transfusion; Cs — cesarean section

Table 5. Neonatal results

Variables Number (%):		Control (n = 54) Number (%):	Case (n = 27) Number (%):	P
NICU	No	53 (98.1)	24 (88.9)	*0.204
	Yes	1 (1.9)	3 (11.1)	
Birth weight	Mean ± SD	3261.06 ± 432.96	2707.96 ± 510.38	** < 0.001
Gestational week	Mean ± SD	38.70 ± 1.34	35.60 ± 2.14	*** < 0.001
APGAR1	Mean ± SD	8.96 ± 0.19	8.59 ± 1.29	***0.532
APGAR5	Mean ± SD	9.96 ± 0.19	9.78 ± 0.64	***0.070

* Chi-Square Test; **t-test, ***Mann-Whitney U; NICU — Neonatal Intensive Care Unit; SD — standard deviation

in a study that examined the 7-year PAS data in a tertiary health institution, although multiparous patients were included, increasing maternal age was found to be an independent risk factor in the development of PAS, which is in line with the results of the present study [21].

Baldwin et al. [14] reported that previous gynecological surgery increased the risk in women diagnosed with primiparous PAS. However, in their study, unlike the present study, gynecological operations were not examined separately, but regardless of the type, it was examined whether there were gynecological operations in the anamnesis. For this reason, a separate risk assessment was not performed for each gynecological operation [14].

In the study conducted by Khander et al. which included 46 PAS cases, the history of cesarean section and myomectomy were compared and it was found that myomectomy was more effective in PAS formation than previous cesarean section [22]. However, the patients who were included in this study were multiparous. In the present study, it was found that previous myomectomy increased PAS formation 11.007-fold.

When the literature data were examined, although few studies report the incidence of primiparous PAS and examine some risk factors, there are not enough data on maternal and neonatal outcomes [14].

In the present study, statistically significant differences were detected in terms of the estimated amount of bleeding, postoperative HB values, need for transfusion, and drain, which is in line with the literature data. The reason for this is serious maternal bleeding during the separation of the placenta in cases complicated with PAS. Although multiparous PAS cases were evaluated in previous studies, it was reported that bleeding during the operation is more common in cases complicated by PAS [5–7].

When the pregnancy results of primiparous PAS cases were examined in the literature, it was found that only the gestational weeks were lower at birth, which is consistent with the results of the present study [14]. The cause of this is that PAS cases undergo cesarean section at the 34–37th gestational weeks [23]. For this reason, the newborn birth weights are statistically less and the gestational weeks at birth are lower.

Limitations

The limitation of the study is that none of the patients who were included in it became pregnant through assisted reproduction method. In addition, PAS cases included in the study do not have long-term results.

Strengths of the study

The strengths of the study are that it was conducted in the largest hospital in the South Marmara region and that all the patients who were included in the study had antenatal follow-up and were elective cases. Also, although there are few studies in the literature investigating the risk factors for PAS in primiparous women, to the best of our knowledge, the present study is the first to evaluate maternal and neonatal outcomes in these women simultaneously.

CONCLUSIONS

PAS is also present in primiparous pregnant women at a considerable rate, and it must be known which patients should be examined in more detail especially in terms of PAS because it is a life-threatening condition in women.

Ethical approval

The approval for the study was obtained from the local ethics committee of a tertiary education and research hospital with the ethics committee number 2011-KAEK-25 2019/08-06. Also, the study was conducted in line with the Declaration of Helsinki principles.

Conflict of interest

There is no conflict of interest.

REFERENCES

1. Oyelese Y, Smulian JC. Placenta previa, placenta accreta, and vasa previa. Obstet Gynecol. 2006; 107(4): 927–941, doi: 10.1097/01.AOG.0000207559.15715.98, indexed in Pubmed: 16582134.
2. Tantbirojn P, Crum CP, Parast MM. Pathophysiology of placenta creta: the role of decidua and extravillous trophoblast. Placenta. 2008; 29(7): 639–645, doi: 10.1016/j.placenta.2008.04.008, indexed in Pubmed: 18514815.
3. Khong TY. The pathology of placenta accreta, a worldwide epidemic. J Clin Pathol. 2008; 61(12): 1243–1246, doi: 10.1136/jcp.2008.055202, indexed in Pubmed: 18641410.
4. Laoreti A, Thilaganathan B, Kaelin Agten A, et al. Placental thickness in the lower uterine segment and invasive placentation: Will the promise live up? Acta Obstet Gynecol Scand. 2019; 98(2): 266, doi: 10.1111/aogs.13460, indexed in Pubmed: 30218616.
5. Young BC, Nadel A, Kaimal A. Does previa location matter? Surgical morbidity associated with location of a placenta previa. J Perinatol. 2014; 34(4): 264–267, doi: 10.1038/jp.2013.185, indexed in Pubmed: 24480901.
6. Altinkaya SO, Nergiz S, Guldas M, et al. Antepartum risk factors for peripartum hysterectomy in women with placenta previa. Gynecol Obstet Reprod Med. 2016; 20(01): 24–28.
7. Sentilhes L, Kayem G, Silver RM. Conservative Management of Placenta Accreta Spectrum. Clin Obstet Gynecol. 2018; 61(4): 783–794, doi: 10.1097/GRF.0000000000000395, indexed in Pubmed: 30222610.
8. Badr DA, Al Hassan J, Salem Wehbe G, et al. Uterine body placenta accreta spectrum: A detailed literature review. Placenta. 2020; 95: 44–52, doi: 10.1016/j.placenta.2020.04.005, indexed in Pubmed: 32452401.
9. Grobman WA, Gersnoviez R, Landon MB, et al. National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units (MFMU) Network, National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network, National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Maternal morbidity associated with multiple repeat cesarean deliveries. Obstet Gynecol. 2006; 107(6): 1226–1232, doi: 10.1097/01.AOG.0000219750.79480.84, indexed in Pubmed: 16738145.
10. Farquhar CM, Li Z, Lensen S, et al. Incidence, risk factors and perinatal outcomes for placenta accreta in Australia and New Zealand: a case-control study. BMJ Open. 2017; 7(10): e017713, doi: 10.1136/bmjopen-2017-017713, indexed in Pubmed: 28982832.
11. Senkoro EE, Mwanamsangu AH, Chuwa FS, et al. Frequency, Risk Factors, and Adverse Fetomaternal Outcomes of Placenta Previa in Northern Tanzania. J Pregnancy. 2017; 2017: 5936309, doi: 10.1155/2017/5936309, indexed in Pubmed: 28321338.
12. Baldwin HJ, Patterson JA, Nippita TA, et al. Maternal and neonatal outcomes following abnormally invasive placenta: a population-based record linkage study. Acta Obstet Gynecol Scand. 2017; 96(11): 1373–1381, doi: 10.1111/aogs.13201, indexed in Pubmed: 28805942.
13. Lindqvist PG, Thurn L, Thurn L, et al. Abnormally invasive placenta-prevalence, risk factors and antenatal suspicion: results from a large population-based pregnancy cohort study in the Nordic countries. BJOG. 2016; 123(8): 1348–1355, doi: 10.1111/1471-0528.13547, indexed in Pubmed: 26227006.
14. Baldwin HJ, Patterson JA, Nippita TA, et al. Antecedents of Abnormally Invasive Placenta in Primiparous Women: Risk Associated With Gynecologic Procedures. Obstet Gynecol. 2018; 131(2): 227–233, doi: 10.1097/AOG.0000000000002434, indexed in Pubmed: 29324602.
15. World Health Organization (Trends In Maternal Mortality 2000 to 2017: Estimates by WHO, UNICEF, UNFPA, World Bank Group, and the United Nations Population Division Geneva, 2019.
16. Iacovelli A, Liberati M, Khalil A, et al. Risk factors for abnormally invasive placenta: a systematic review and meta-analysis. J Matern Fetal Neonatal Med. 2020; 33(3): 471–481, doi: 10.1080/14767058.2018.1493453, indexed in Pubmed: 29938551.
17. Fitzpatrick KE, Sellers S, Spark P, et al. Incidence and risk factors for placenta accreta/increta/percreta in the UK: a national case-control study. PLoS One. 2012; 7(12): e52893, doi: 10.1371/journal.pone.0052893, indexed in Pubmed: 23300807.
18. Miller DA, Chollet JA, Goodwin TM. Clinical risk factors for placenta previa-placenta accreta. Am J Obstet Gynecol. 1997; 177(1): 210–214, doi: 10.1016/s0002-9378(97)70463-0, indexed in Pubmed: 9240608.
19. Jacques SM, Qureshi F, Trent VS, et al. Placenta accreta: mild cases diagnosed by placental examination. Int J Gynecol Pathol. 1996; 15(1): 28–33, doi: 10.1097/00004347-199601000-00005, indexed in Pubmed: 8852443.
20. Pron G, Mocarski E, Bennett J, et al. Ontario UFE Collaborative Group. Pregnancy after uterine artery embolization for leiomyomata: the Ontario multicenter trial. Obstet Gynecol. 2005; 105(1): 67–76, doi: 10.1097/01.AOG.0000149156.07061.1f, indexed in Pubmed: 15625144.
21. Salmanian B, Fox KA, Arian SE, et al. In vitro fertilization as an independent risk factor for placenta accreta spectrum. Am J Obstet Gynecol. 2020; 223(4): 568.e1–568.e5, doi: 10.1016/j.ajog.2020.04.026, indexed in Pubmed: 32360847.
22. Khander A, Sharma N, Eroglu I,et al. Ultrasound detection rates of the placenta accreta spectrum with prior myomectomy. J Matern Fetal Neonatal Med. 2022 Dec;35(25):8752-8755, doi: 10.1080/14767058.2021.2001800, indexed in Pubmed: 34763606.
23. Donovan BM, Shainker SA. Placenta Accreta Spectrum. Neoreviews. 2021; 22(11): e722–e733, doi: 10.1542/neo.22-11-e722, indexed in Pubmed: 34725137.

VIA MEDICA

ORIGINAL PAPER / OBSTETRICS

Ginekologia Polska
2023, vol. 94, no. 2, 141–145

ISSN 0017–0011, e-ISSN 2543–6767

DOI 10.5603/GP.a2023.003

Effect of the implementation of an enhanced recovery after surgery protocol (ERAS) in patients undergoing an elective cesarean section

Maria Ofelia Sordia-Pineyro[1], Carlos Villegas-Cruz[1], Magdalena Hernandez-Bazaldua[1], Alfredo Pineyro-Cantu[1], Tracy Gaston-Locsin[1], Luis Humberto Sordia-Hernandez[2]

[1]TEC Salud, Ignacio Morones Prieto, Monterrey, Mexico

[2]Department of Gynecology and Obstetrics, University Hospital "Dr. José E. González", Autonomous University of Nuevo León, Monterrey N. L., Mexico

ABSTRACT

Objectives: To demonstrate that the application of an enhanced recovery after surgery (ERAS) protocol in elective cesarean sections is associated with reduced hospital stay without increasing maternal complications.

Material and methods: This retrospective, comparative study included patients who underwent an elective cesarean section. The patients were divided into groups: group 1, women who received elements of standardized care according to ERAS guidelines, and group 2, women who did not receive this care.

Results: The study included 295 patients, 139 in group 1 (ERAS) and 156 in group 2. The demographic characteristics were similar. Hospital stay and postoperative pain at 24 and 48 hours were lower in patients in group 1; these differences were statistically significant ($p < 0.001$). The overall complication rate, head pain, surgical wound infection, urinary retention, and readmission were similar in both groups.

Conclusions: The application of an ERAS protocol can reduce hospital stay and postoperative pain without increasing the postoperative complication rate in patients who undergo an elective cesarean section. In developing countries, the application of this protocol could help in optimizing available health system resources.

Key words: ERAS; enhanced recovery; enhanced recovery after surgery; c-section; cesarean section

Ginekologia Polska 2023; 94, 2: 141–145

INTRODUCTION

A cesarean section is a surgical procedure to deliver a fetus and its membranes through a laparotomy and an incision in the uterus (hysterotomy) [1].

Cesarean section is the most common surgery performed worldwide, and its prevalence has increased, increasing the risks of maternal morbidity, and mortality. It is estimated that approximately 18.5 million cesarean sections are performed each year. The increase in cesarean sections in Mexico has been a motive of concern for the government health system. Between 2000 and 2012, the number of cesarean deliveries increased by 50.3%. There is also a clear difference in the number of cesarean deliveries practiced in the public and private practice (40.9% vs 69.9% of all deliveries, respectively). Mexico has the fourth-highest rate of cesarean deliveries in the world [2, 3].

It is estimated that up to 45% of all cesarean deliveries are electively programmed. This surgical procedure increases hospital stay, and therefore, the cost of medical care compared to vaginal deliveries. The most frequent complications of cesarean delivery are bleeding, intrabdominal organ damage, postsurgical infection, and venous thromboembolism [4].

Enhanced recovery after surgery (ERAS) is a multimodal, multidisciplinary concept based on scientific evidence [5]. These guidelines were designed for patients who undergo different surgical procedures. Improved recovery has as its central philosophy to reduce the harmful effects

Corresponding author:
Luis Humberto Sordia-Hernandez
Department of Gynecology and Obstetrics, University Hospital "Dr. José E. González", Autonomous University of Nuevo León, Monterrey N. L., Mexico
e-mail: drsordia@gmail.com

Received: 4.01.2022 Accepted: 27.12.2022 Early publication date: 12.01.2023

of surgery. This allows a rapid and beneficial postsurgical recovery [6]. The most important aspects of this approach are adequate postoperative nutrition, a reduction in the surgical stress response, minimum use of opioids in postsurgical analgesia, early mobilization, and the application of postoperative care designed and managed by a multidisciplinary team [7].

This study aimed to evaluate the postoperative results after applying enhanced recovery after surgery in a group of patients undergoing elective cesarean section in a tertiary-care private hospital in Monterrey, Nuevo Leon, Mexico.

Objectives

To demonstrate that the application of an enhanced recovery after surgery (ERAS) protocol in elective cesarean sections is associated with reduced hospital stay without increasing maternal complications.

MATERIAL AND METHODS

This was a retrospective, comparative study carried out at the Hospital San Jose of the Tec Salud System of the Instituto Tecnológico y de Estudios Superiores de Monterrey in Monterrey, Mexico. Pregnant women between 18 and 40 years of age with an electively planned cesarean section from January 1, 2017, to December 31, 2020, were included with previous Ethics Committee approval (P000237-AGECE 120-CI-CR002).

Inclusion criteria were women 18 to 40 years of age with a term pregnancy (37 to 41 weeks of gestation) programmed for an elective cesarean section. The exclusion criteria were patients undergoing an emergency cesarean section, women with obstetric hemorrhage, patients with active labor, infection, a hysterotomy other than Kerr, with more than three previous cesarean sections, morbid obesity [body mass index (BMI) > 40 m^2/kg], placentation disorders, a history of hypertension and/or diabetes mellitus, hypertensive disease of pregnancy, and/or gestational diabetes, women with kidney function abnormalities, and patients with platelet and/or coagulation disorders. Patients with incomplete medical records were excluded.

The patients were divided into groups. Group 1, patients undergoing an elective cesarean section during 2020 and who received a series of standardized preoperative, perioperative, and postoperative care according to ERAS guidelines implemented in the hospital, and group 2, patients who underwent an elective cesarean section during 2017 and 2018 before the implementation of ERAS care in the hospital. All procedures from both groups of patients were performed by the same group of surgeons.

The applied standardized procedures consisted of not performing bowel preparation before the procedure, allowing the intake of fluids and carbohydrates up to six hours before surgery, not using pre-anesthetic sedation, thromboprophylaxis with knee compression stockings, a prophylactic antibiotic (cephalothin 2 grams intravenously 30 to 60 minutes before the skin incision; in the case of penicillin allergy, gentamicin 80 mg intravenously), no pubic hair shaving, and a surgical scrub with chlorhexidine.

A Kerr-type hysterotomy was performed, a liquid diet was started early after surgery (4 h), intravenous fluids were administrated for a maximum of 24 hours after surgery, nausea and vomiting were controlled pharmacologically, and postoperative pain control was made paracetamol and ketorolac, opioids were avoided as much as possible. The urinary catheter was removed, and ambulation was started 12 h after surgery. A visual analog scale was used to assess postoperative pain. All the complications observed during the study were reported.

Statistical analysis was performed with the Kolmogorov-Smirnov test to determine the normality of the variables. Categorical variables were reported as frequencies and percentages and continuous variables with a non-normal distribution as medians and ranges. The characteristics of both groups were compared using the Mann-Whitney U test for continuous variables and Pearson's chi-square or Fisher's exact test for categorical variables. All tests were bilateral, considering a p value < 0.05 as statistically significant. SPSS statistical software version 16 was used to analyze the data.

RESULTS

A total of 295 patients were subjected to the surgical procedure. Group 1 included 139 patients who received the previously described care according to ERAS guidelines. Group 2 included 156 patients who had an elective cesarean section but without applying the described standardized procedures.

The women who underwent the ERAS protocol were older (30.5 ± 5.2 vs 29.1 ± 4.8), and this difference was statistically significant ($p = 0.01$). The BMI was similar in both groups (29.9 kg/m^2 vs 30.6 kg/m^2; $p = 0.08$). The marital status of the patients, height and BMI are summarized in Table 1. The gestational age at surgical intervention was similar in both groups (Tab. 1).

All the women included, on both groups, received a Kerr hysterotomy, thromboprophylaxis, and antibiotic prophylaxis. Intravenous fluids with Hartmann's and 5% glucose solutions were provided to all patients. Hartman's solution was administered during the surgical procedure. After delivery, 5% glucose solution and Hartman solution were administered alternately.

Most of the women in both groups received some preoperative sedation (72.3%), an antiemetic (81.2%),

Table 1. Demographic characteristics of the patients

Characteristic	Group 1 (139, 47.2%)	Group 2 (156, 52.9%)	p value
Age [years]	30.5 ± 5.2 (19–44)	29.1 ± 4.8 (18–44)	0.018
Weight [kg]	75.7 ± 12.0 (51–112)	78.3± 11.8 (48–105)	0.037
Height [m]	1.59 ± 6.3 (1.43–1.76)	1.60 ± 5.7 (1.47–1.74)	0.38
BMI [kg/m^2]	29.9 ± 4.2 (18.3–38.7)	30.6 ± 4.8 (20.2–39.5)	0.08
Gestational age [weeks]	38.5 ± 0.85 (37–40.1)	38.5 ± 0.85 (37–41.3)	0.72
Marital status, married, n (%)	136 (87.2)	127 (90.7)	0.48

Data are presented as means ± standard deviation and (ranges) unless otherwise stated; BMI — body mass index

Table 2. Comparison of duration times of interventions in the groups

Intervention	Group 1 n = 139	Group 2 n = 156	p value
Fast [hours]	8.0 ± 1.9 (3–20)	11.3 ± 2.31 (2–24)	< 0.001
Intravenous solutions [hours]	20.7 ± 4.1 (8–24)	19.9 ± 4.3 (12–24)	0.15
Pain scale points [24 hours]	2.8 ± 2.1 (1–10)	4.0 ± 1.3 (1–10)	< 0.001
Pain scale points [48 hours]	2.1 ± 1.2 (1–9)	2.8 ± 1.9 (1–10)	> 0.99
Removal of urinary catheter [hours]	20.1 ± 4.4 (10–55)	22.3 ± 4.6 (12–44)	< 0.001
Start of oral intake [hours]	8.8 ± 2.9 (2–16)	10.5 ± 2.8 (6–20)	< 0.001
Ambulation [hours]	20.2 ± 3.9 (11–22)	22.6 ± 4.1 (12–44)	< 0.001
Postsurgical hospital stay [hours]	44.0 ± 5.4 (31–60)	50.2 ± 8.2 (37–84)	< 0.001

Data are presented as means ± standard deviation and (ranges) unless otherwise stated

Table 3. Comparison of number and type of complications between the two study groups

	Group 1, n = 139	Group 2, n = 156	p value
Complications	3 (2.1)	4 (2.6)	1
Hospital readmission	1 (0.7)	2 (1.3)	1
Surgical wound infection	1 (0.7)	2 (1.3)	1
Head pain	2 (1.4)	2 (1.3)	1
Urinary retention	1 (0.7)	0	0.47

Some patients had more than one complication

and postoperative opioids (84.8%). There was no significant difference between the groups.

Skin antisepsis was performed with chlorhexidine. Body temperature was strictly monitored. These procedures were not performed in group 2 (patients who did not receive the ERAS protocol). The fasting period in group 1 was shorter than in group 2 (8.0 ± 1.9 vs 11.3 ± 2.3 h). This difference was statistically significant ($p < 0.01$). The urinary catheter was removed (20.1 ± 4.4 vs 22.3 ± 4.6 h), and diet (8.8 ± 2.9 vs 10.5 ± 2.8 h) was started sooner in group 1; ambulation also started sooner (20.2 ± 3.9 vs 22.6 ± 4.1 h). These findings were statistically significant ($p < 0.01$) (Tab. 2). According to the visual analog scale, there was less pain at 24 h in group 1 (2.8 ± 2.1 vs 4.0 ± 1.3). This finding was statistically significant ($p < 0.01$); however, the results of postoperative pain at 48 hours were not significant (2.1 ± 1.2 vs 2.8 ± 1.9) (Tab. 2). The women in group 1 had a shorter hospital stay (44 ± 5.4 vs 50 ± 8.2 hours; $p < 0.01$) and also received less antibiotic therapy (80.7% vs 89.1%; $p < 0.05$).

Only seven patients (2.4%) of all the women included in the study had a complication. The complications observed were hospital readmission (3, 1%), surgical wound infection (3, 1%), head pain (4, 1.4%), and urinary retention (1, 0.3%). Patients who had a surgical wound infection were the ones who were readmitted, one of them also showed urinary retention. There was no significant difference between the two groups regarding complications (2.6% vs 2.1%; $p > 0.9$) (Tab. 3).

DISCUSSION

The implementation of the ERAS protocol in women subjected to an elective cesarean section was associated with a reduction in postoperative pain and time of hospital stay. Likewise, patients in this group started a postoperative diet earlier and had the urinary catheter removed sooner, which allowed early postoperative ambulation. These benefits helped speed up the hospital discharge of these patients.

Some studies have reported a shortened hospital stay and a reduction in surgical complications when this protocol is applied, a situation that reduces total costs for the patient [8, 9]. These benefits translate into quality medical care, generating greater security which the patient perceives as a better surgical experience.

In our study, the complication rate was similar in both groups. Therefore, the interventions in the ERAS protocol do not increase the complication rate and offer the possibility of significantly shortening the hospital stay of patients who undergo an elective cesarean section.

Enhanced recovery after surgery is a series of guidelines developed in 2001 by a group of surgeons in Europe with a multidisciplinary approach that focuses on surgical patients [10]. The ERAS protocol involves several elements of care, such as carbohydrate drinks two hours before surgery, early mobilization, and early oral postoperative intake of fluids and food (the same day of surgery) [5, 11].

The care proposed in the ERAs protocol is divided into specific elements. Preoperatively, carbohydrate fluids are recommended at least two hours before surgery to reduce insulin resistance and increase early recovery, prophylaxis against thrombosis, antibiotics as prophylaxis against infection at least 60 minutes before the skin incision, and antiemetics to reduce the possibility of postoperative nausea and vomiting [11]. Whether they were in the ERAS group or not, all women in our study received prophylaxis against thrombosis and infection; there was no difference in the frequency of administration of antiemetics in the two groups ($p = 0.26$).

The ERAS protocol proposes controlling body temperature using thermal blankets and warmed intravenous solutions to reduce complications in the intraoperative period [12]. In our study, body temperature was controlled in 100% of the patients in the ERAS protocol and none in group 2 ($p < 0.001$).

Finally, in the postoperative period, several measures are recommended. First, early mobilization and early oral intake of fluids and solids on the day of surgery to reduce the insulin resistance induced by fasting; early removal of the urinary catheter and intravenous fluids; pain management limiting opioid administration; and preparation for early discharge [13]. In our study, there were no significant differences between the hours of intravenous solution administration ($p = 0.15$). The urinary catheter was removed earlier in women in the ERAS protocol; an early oral diet after surgery and early ambulation also occurred in the ERAS patients. However, the most relevant aspect of our results was undoubtedly the reduction in hospital stay after surgery in the patients in the ERAS group. In any health system, a decrease in the length of hospital stay represents resource savings that allow a greater number of women to be cared for more efficiently.

Some data suggest that the implementation of the ERAS protocol can reduce complications by 10 to 20% [14, 15]. In this study, the number of complications did not differ between the two groups. The sample size seen in the study did not allow a statistical comparison of complication rates.

The ERAS protocol has shown to reduce hospital length stay, in our study the difference was statistically significant between both groups, since the difference was six hours, it may not appear to be clinically relevant for a single patient but applied on public health system on a single hospital in our city that has 4800 cesarean sections per year that is 28,800 less hours.

The ERAS protocol also reduces health costs [16]. For example, a study published by Rhou et al. [17] reported that the costs of patients who underwent laparoscopic hysterectomy with the ERAS protocol were lower than those of patients undergoing the same surgery but who did not follow the ERAS protocol ($p = 0.001$). This data is important. Our population could benefit because Mexico is considered a developing country.

One of the limitations of the present study is the fact that some of the procedures that are part of the ERAS protocol may be similar to the procedures that are conventionally used in clinical practice.

CONCLUSIONS

The application of an ERAS protocol can reduce hospital stay and postoperative pain without increasing the postoperative complication rate in patients who undergo an elective cesarean section. In developing countries, the application of this protocol could help optimize available health system resources.

Ethics

Ethics Committee approval number: P000237-AGECE 120-CI-CR002.

Acknowledgments

We thank Sergio Lozano-Rodriguez for his help in translating and editing the manuscript.

Funding

This study did not receive funding from the public, commercial or not-for-profit sectors.

Conflict of interests

The authors declare that they have no conflicts of interest.

REFERENCES

1. Maskey S, Bajracharya M, Bhandari S. Prevalence of cesarean section and its indications in a tertiary care hospital. JNMA J Nepal Med Assoc. 2019; 57(216): 70–73, doi: 10.31729/jnma.4282, indexed in Pubmed: 31477935.
2. Dahlke JD, Mendez-Figueroa H, Rouse DJ, et al. Evidence-based surgery for cesarean delivery: an updated systematic review. Am J Obstet Gynecol. 2013; 209(4): 294–306, doi: 10.1016/j.ajog.2013.02.043, indexed in Pubmed: 23467047.
3. Suárez L, Campero L, de la Vara E, Rivera L, Hernández M, Walker D, et al. High recurrence of cesarean sections: reversing the trend and improving the quality of delivery. National Health and Nutrition Survey 2012. Mexico City: National Institute of Public Health. 2012.
4. van Ham MA, van Dongen PW, Mulder J. Maternal consequences of caesarean section. A retrospective study of intra-operative and postoperative maternal complications of caesarean section during a 10-year period. Eur J Obstet Gynecol Reprod Biol. 1997; 74(1): 1–6, doi: 10.1016/s0301-2115(97)02725-5, indexed in Pubmed: 9243191.
5. Ljungqvist O, Scott M, Fearon KC. Enhanced recovery after surgery: a review. JAMA Surg. 2017; 152(3): 292–298, doi: 10.1001/jamasurg.2016.4952, indexed in Pubmed: 28097305.
6. Slim K, Delaunay L, Joris J, et al. Le Groupe francophone de réhabilitation améliorée après chirurgie (GRACE). How to implement an enhanced recovery program? Proposals from the Francophone Group for enhanced recovery after surgery (GRACE). J Visc Surg. 2016; 153(6S): S45–S49, doi: 10.1016/j.jviscsurg.2016.05.008, indexed in Pubmed: 27316295.
7. Wrench IJ, Allison A, Galimberti A, et al. Introduction of enhanced recovery for elective caesarean section enabling next day discharge: a tertiary centre experience. Int J Obstet Anesth. 2015; 24(2): 124–130, doi: 10.1016/j.ijoa.2015.01.003, indexed in Pubmed: 25794417.
8. Senturk JC, Kristo G, Gold J, et al. The development of enhanced recovery after surgery across surgical specialties. J Laparoendosc Adv Surg Tech A. 2017; 27(9): 863–870, doi: 10.1089/lap.2017.0317, indexed in Pubmed: 28795911.
9. Ban KA, Berian JR, Ko CY. Does implementation of Enhanced Recovery After Surgery (ERAS) protocols in colorectal surgery improve patient outcomes? Clin Colon Rectal Surg. 2019; 32(2): 109–113, doi: 10.1055/s-0038-1676475, indexed in Pubmed: 30833859.
10. Tanious MK, Ljungqvist O, Urman RD. Enhanced recovery after surgery: history, evolution, guidelines, and future directions. Int Anesthesiol Clin. 2017; 55(4): 1–11, doi: 10.1097/AIA.0000000000000167, indexed in Pubmed: 28901977.
11. Nelson G, Altman AD, Nick A, et al. Guidelines for pre- and intra-operative care in gynecologic/oncology surgery: Enhanced Recovery After Surgery (ERAS®) Society recommendations--Part I. Gynecol Oncol. 2016; 140(2): 313–322, doi: 10.1016/j.ygyno.2015.11.015, indexed in Pubmed: 26603969.
12. Macones GA, Caughey AB, Wood SL, et al. Guidelines for postoperative care in cesarean delivery: Enhanced Recovery After Surgery (ERAS) society recommendations (part 3). Obstetric Anesthesia Digest. 2020; 40(2): 69–70, doi: 10.1097/01.aoa.0000661356.57542.dd.
13. Caughey AB, Wood SL, Macones GA, et al. Guidelines for intraoperative care in cesarean delivery: Enhanced Recovery After Surgery society recommendations (part 2). Am J Obstet Gynecol. 2018; 219(6): 533–544, doi: 10.1016/j.ajog.2018.08.006, indexed in Pubmed: 30118692.
14. Gustafsson UO, Hausel J, Thorell A, et al. Enhanced Recovery After Surgery Study Group. Adherence to the enhanced recovery after surgery protocol and outcomes after colorectal cancer surgery. Arch Surg. 2011; 146(5): 571–577, doi: 10.1001/archsurg.2010.309, indexed in Pubmed: 21242424.
15. ERAS Compliance Group. The Impact of Enhanced Recovery Protocol Compliance on Elective Colorectal Cancer Resection: Results From an International Registry. Ann Surg. 2015; 261(6): 1153–1159, doi: 10.1097/SLA.0000000000001029, indexed in Pubmed: 25671587.
16. Fay EE, Hitti JE, Delgado CM, et al. An enhanced recovery after surgery pathway for cesarean delivery decreases hospital stay and cost. Am J Obstet Gynecol. 2019; 221(4): 349.e1–349.e9, doi: 10.1016/j.ajog.2019.06.041, indexed in Pubmed: 31238038.
17. Rhou YJJ, Pather S, Loadsman JA, et al. Direct hospital costs of total laparoscopic hysterectomy compared with fast-track open hysterectomy at a tertiary hospital: a retrospective case-controlled study. Aust N Z J Obstet Gynaecol. 2015; 55(6): 584–587, doi: 10.1111/ajo.12093, indexed in Pubmed: 23634987.

VIA MEDICA

ORIGINAL PAPER / OBSTETRICS

Ginekologia Polska
2023, vol. 94, no. 2, 146–151

ISSN 0017–0011, e-ISSN 2543–6767

DOI 10.5603/GP.a2022.0046

Use of the expanded Apgar score for the assessment of intraventricular and intraparenchymal haemorrhage risk in neonates

Agnieszka Goralska[1], Joanna E. Puskarz-Gasowska[1], Pawel Bujnowski[2], Renata Bokiniec[1]

[1]Neonatal and Intensive Care Department, Medical University of Warsaw, Poland
[2]Systems Research Institute of the Polish Academy of Sciences, Warsaw, Poland

ABSTRACT

Objectives: Preterm birth is a key factor contributing to haemorrhage incidence in neonates. This study focused on defining relevant parameters for the assessment of intraventricular and intraparenchymal haemorrhage risks in neonates.

Material and methods: Chi-square automatic interaction detection was used to analyse the Apgar score (AS), the Apgar max score, and the course of resuscitation documented according to the expanded AS in 696 infants born between 2009 and 2011 in the Neonatal and Intensive Care Department of the Medical University of Warsaw.

Results: Gestational age was the most relevant discriminating variable for the prediction of intraventricular III degree and intraparenchymal haemorrhage incidences. Infants born before the 31st week of pregnancy made up 80% of the intraventricular or intraparenchymal haemorrhage cases. Additionally, a fraction of inspired oxygen > 0.8 at ten minutes after birth was a better discriminating variable in the youngest neonates than an Apgar max score ≤ 5, identifying 31.6% and 20.6% of infants with intraventricular and intraparenchymal haemorrhage, respectively.

Conclusions: Consideration of the oxygen concentration supplied during resuscitation significantly improves the prognosis of intraventricular and intraparenchymal haemorrhages in preemies compared to the use of the classical AS.

Key words: algorithm; Apgar score; CHIAD; IPH; IVH

Ginekologia Polska 2023; 94, 2: 146–151

INTRODUCTION

In 1952, in response to a student who asked her how to estimate the health status of a newborn immediately after birth, Virginia Apgar wrote down five points on a piece of tissue paper. A year later, the Apgar score (AS) was made public, and two years later it was officially published. In 2015, the *American Academy of Paediatrics* (AAP) and the American College of Obstetricians and Gynaecologists (ACOG) accepted the AS as a reliable and convenient measure of an infant's health status and the reaction to resuscitation, if needed [1, 2].

The AS was never intended to serve as a prognostic tool, and according to the *AAP and the ACOG guidelines*, it should not be used to predict individual neonatal mortality or neurologic outcome. However, the correlation between the AS and a newborn's further development became of great interest in clinical practice [1–5]. A low AS in the fifth minute after birth has been linked to abnormal development later in life [6, 7]. Numerous studies also indicate an impact of the procedures performed immediately after birth on the incidence of retinopathy of prematurity (ROP), bronchopulmonary dysplasia (BPD), and intraventricular or intraparenchymal haemorrhage (IVH/IPH), particularly in preemies [8–10]. In fact, the immaturity of preemies is the main cause of IVH III and IPH [11, 12]. IVH III and IPH substantially contribute to further complications and neonatal mortality. They are also key causes of *developmental disorders of motor*, cognitive, and behavioural functions in schoolchildren [12].

Since an infant's condition and further development often depends on medical interventions used to support postnatal transition, a description of these procedures is an important component of an alternative scoring system

Corresponding author:
Renata Bokiniec
Neonatal and Intensive Care Department, Medical University of Warsaw, Karowa 2, 00-315 Warsaw, Poland
e-mail: r.bokiniec@wp.pl

Received: 14.06.2021 Accepted: 11.04.2022 Early publication date: 3.06.2022

— the extended Apgar score. The extended AS rates the same five signs as the original AS (heart rate, skin colour, muscle tone, reflex irritability, and respiratory function) but extends the observations and scoring to 10, 15, and 20 minutes after birth (if the 5-minute AS is < 7) [13]. Additionally, the expanded AS allows the resuscitative procedures performed immediately after birth to be recorded, including continuous positive airway pressure, oxygen supplementation bag and mask ventilation, intubation and ventilation, chest compressions, and surfactant and/or epinephrine administration [13].

Objectives

In the present study, using the expanded AS, we investigated if the interventions performed in the labour ward can reliably predict the incidence of early complications in infancy. In particular, we focussed on the incidence of IVH III and IPH in neonates, as they have the highest clinical impact.

MATERIAL AND METHODS

Our single-centre study was based on the medical documentation of 696 infants born between 2009 and 2011 in the Neonatal and Intensive Care Department Medical University of Warsaw, which was sufficient to perform statistical analyses. This group comprised 276 infants (147 boys and 129 girls) born between the 23rd and 31st week of gestational age (GA), 190 infants (100 boys and 90 girls) born between the 32nd and 36th week of GA, and 230 infants (119 boys and 111 girls) born after the 37th week of GA. The study included neonates that required resuscitation in the first minutes after birth (ventilation using a mask ventilator, intubation, oxygen concentration used, cardiac massage, and epinephrine use) and with an AS ≤ 7 in the first minute after birth. The exclusion criteria included an AS > 7, the presence of significant congenital disorders, and a lack of resuscitation interventions. The youngest group (≤ 31st week GA) studied in detail consisted of 109 infants born by vaginal birth and 167 by caesarean section, with an average duration of pregnancy of 27.8 weeks.

The following analysed parameters were used as inputs for the chi-square automatic interaction detection (CHAID) algorithm: i) the AS at 1, 3, 5, 10, and 15 minutes after birth; ii) the maximum value from the AS measured at 1, 3, 5, and 10 minutes after birth (AS max); and iii) the course of resuscitation documented according to the expanded AS (ventilation using a mask ventilator, intubation, oxygen concentration used, cardiac massage, and epinephrine use).

Statistical analysis

The CHAID algorithm, which assures high specificity and sensitivity of the analysis and statistically significant division of the nodes, was used for data analysis (SPSS 15.0). Data were compared using Pearson's chi-square test with p set at 0.05 (Bonferroni adjusted for multiple comparisons) and a cut-off selected automatically for all the parameters. The algorithm starts with the discretisation of the continuous variables. Next, the variable that best discriminates between decision classes is chosen based on the results of the chi-square test. In this way, the whole set of observations (*i.e.*, root node) is split into subsets (*i.e.*, internal nodes) that are as homogeneous as possible (regarding decision classes), with the branches extending from the root to the nodes describing the rules of division. The internal nodes are then further divided based on the results of the chi-square test. The division continues until nodes containing only one decision class (*i.e.*, leaf nodes) are obtained, the desired depth of the tree is reached, or when the outcome of the next division does not significantly change the structure of the decision classes. Optionally, the decision tree can be trimmed. The branches (rules of division) that do not significantly influence the accuracy to distinguish between decision classes regarding preceding nodes can be shortened.

The branches linking the nodes, going from the top (root) to the bottom (leaves) of the tree, create decision rules in the form of implications: if A and if B and if… and if Z, then the ratio of the decision class (*e.g.*, incidence of IVH) equals X. We aimed to define the relevant parameters affecting IVH III/IPH incidence and to assess the risk of IVH III/IPH based on medical findings and their interactions. Therefore, the classes of our binary variable, namely the presence or lack of IVH III/IPH, were marked as 1 or 0, respectively.

RESULTS

To evaluate the relationship between IVH III/IPH incidence and gestational age, we first prepared a frequency plot (Fig. 1). IVH III cases were observed most frequently in the youngest neonates. Because 80% of IVH III/IPH cases were observed in the infants born before the 31st week of GA, only these neonates were chosen for further analysis.

Artificial intelligence algorithms are currently the tool of choice in medical diagnostics and in decision-making systems. We used one of these modern statistical tools — a CHAID decision tree — to create a clear knowledge system based on decision rules. Next, we constructed a CHAID decision tree. Based on the CHAID algorithm, the expanded AS and a fraction of inspired oxygen (FiO_2) > 0.8 at 10 minutes after birth were chosen as variables with the highest discriminating power. By using these parameters in the analysis, we identified 12 infants with IVH III/IPH in a group of 38 neonates (31.6%) (Fig. 2). When we used the classical AS and an AS max ≤ 5, we identified 13 neonates with IVH III/IPH in a group of 63 infants (20.6%) (Fig. 3).

These results indicate a higher discriminative power of the FiO_2 at 10 minutes after birth (based on the expanded

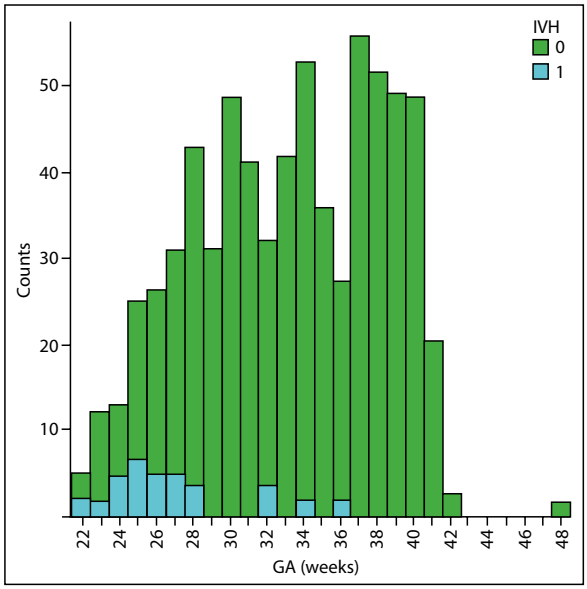


Figure 1. Intraventricular or intraparenchymal haemorrhage (IVH III/IPH) frequency distribution by pregnancy week; The frequency of IVH III/IPH is shown in relation to gestational age (in weeks). For every gestational week, the number of infants with (1, blue) or without (0, red) IVH III/IPH is shown. n = 696

AS) than the AS max. In the key leaf node, the discriminative power of the expanded AS to identify infants with IVH III/IPH was 10% higher than that of the AS max. Additionally, using the expanded AS resulted in a more specific (*i.e.*, smaller) subgroup of neonates (38 vs 63), in which a similar number of IVH III/IPH cases was identified (12 vs 13).

The above analysis was conducted on the group of youngest infants (≤ 31 weeks GA), who experienced IVH III/IPH the most often. In this group, an $FiO_2 > 0.8$ at 10 minutes after birth was a discriminating parameter. Therefore, we decided to investigate this parameter further. We analysed the whole group of infants and added the GA as a new parameter to our decision tree. In this way, the tree reached a depth of two. In the node of ≤ 26 weeks of GA, a new division was generated, creating two subgroups based on the FiO_2 at 10 minutes after birth as a decision parameter: ≤ 0.3 and > 0.3. An $FiO_2 > 0.3$ was a discriminating value in this analysis and identified 29.1% of the group as having experienced IVH III/IPH (Fig. 4). Lastly, using an $FiO_2 \geq 21\%$ as a reference point, we analysed the concentration of oxygen that was given to the infants with IVH III/IPH (Fig. 5). An $FiO_2 > 80\%$ resulted in the highest IVH III/IPH incidence. An $FiO_2 > 30\%$ identified IVH III/IPH cases less precisely, but it included a larger group of infants. Therefore, even though we suggest the FiO_2 as a powerful discriminative parameter, a direct correlation between the oxygen concentration and IVH III/IPH incidence should be evaluated carefully and might require further analysis of, for example, the partial pressures of carbon dioxide (pCO_2) and oxygen (pO_2).

DISCUSSION

The objective evaluation of an infant's health status is important not only for the care the newborn requires but also for scientific research. The need for an unambiguous and comparable record of such an assessment led to the invention of a clear numerical scale. Virginia Apgar was

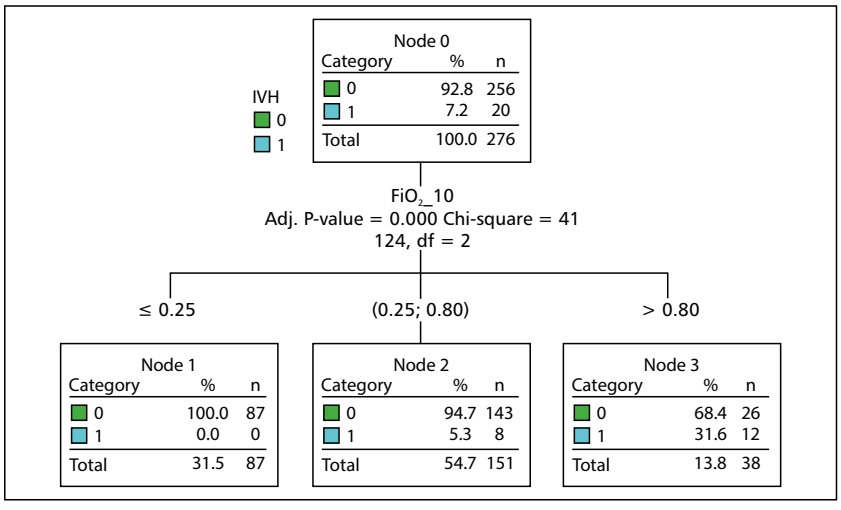


Figure 2. The decision tree based on the expanded Apgar score (fraction of inspired oxygen 10 minutes after birth) for infants ≤ 31st week of gestational age (GA); The group consisting of the youngest infants (≤ 31st week of GA) was split into three subgroups based on the fraction of inspired oxygen (FiO_2) at 10 minutes after birth: ≤ 0.25, 0.25–0.8, and > 0.8. The decision classes referring to the presence or lack of IVH III/IPH were marked as 1 or 0, respectively. n = 276; Pearson's chi-square test with P inset at 0.05 (Bonferroni adjusted for multiple comparisons) was used to assess statistical significance

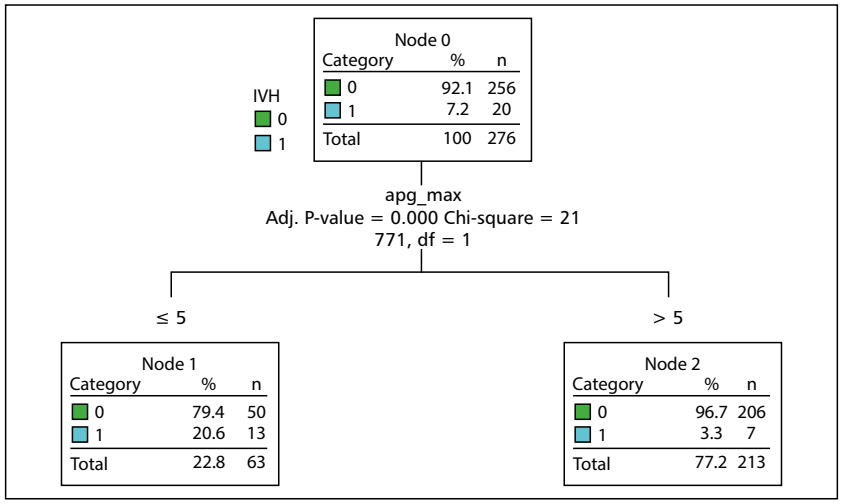


Figure 3. The decision tree based on Apgar max score; The group consisting of the youngest infants (≤ 31st week of GA) was split into two groups based on the Apgar max score (apg_max): ≤ 5 and > 5. The decision classes referring to the presence or lack of IVH III/IPH were marked as 1 or 0, respectively. n = 276; Pearson's chi-square test with p inset at 0.05 (Bonferroni adjusted for multiple comparisons) was used to assess statistical significance

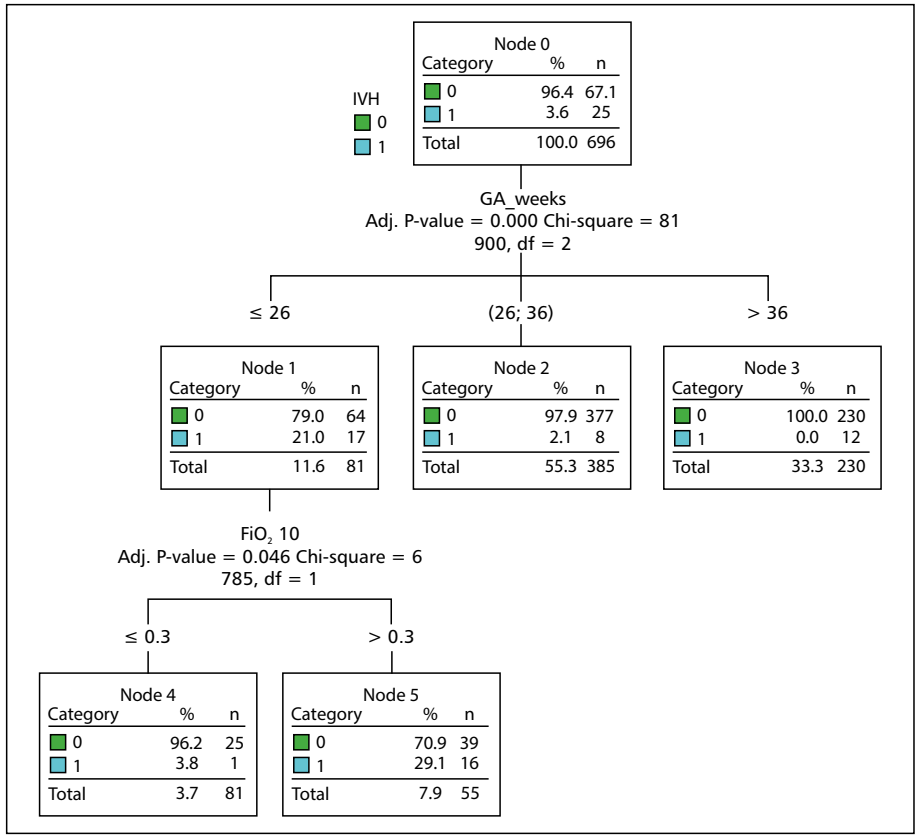


Figure 4. The decision tree relating IVH III/IPH incidence to the gestational age (GA) and the fraction of inspired oxygen (FiO_2) ten minutes after birth; The study group (n = 696) in the root node was split into three subgroups based on GA: ≤ 26th, 26th–36th, and > 36th week of GA. The node ≤ 26th week of GA was further divided into subgroups according to the FiO_2 ten minutes after birth: ≤ 0.3 and > 0.3. The decision classes referring to the presence or lack of IVH III/IPH were marked as 1 or 0, respectively. Pearson's chi-square test with p inset at 0.05 (Bonferroni adjusted for multiple comparisons) was used to assess statistical significance

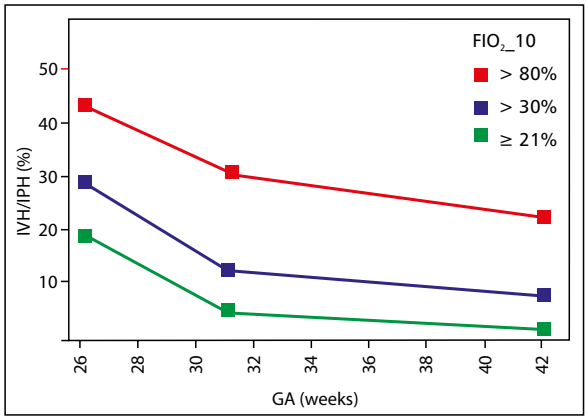


Figure 5. Effect of the fraction of inspired oxygen (FiO_2) ten minutes after birth on IVH III/IPH incidence according to gestational age (GA); The percentages (%) of cumulative IVH III/IPH incidence depending on the FiO_2 ten minutes after birth were plotted against GA: > 80% (marked in red), > 30% (marked in blue), and ≥ 21% (marked in green). Squares indicate data points: GA ≤ 26, GA ≤ 31, and GA ≤ 42. The dashed lines represent estimations of the values. FiO_2 > 80% shows the highest penetration of IVH III/IPH incidence. FiO_2 > 30% identifies IVH III/IPH incidences less precisely but includes a larger group of infants. FiO_2 ≥ 21% was used as a reference point

the first to propose a measure of an infant's health status that is quantitative, and therefore applicable in medical research.

To assess the health status of infants born prematurely and those requiring medical interventions, Rudiger et al. [13, 14] suggested a modification of the AS (the specified AS). The modification aimed to relate the AS to the GA, but it was independent of the requirements to achieve the scor. Hence, an infant born full-term or prematurely, who has no problems adapting to extrauterine life or who reacts favourably to resuscitation or other interventions, obtains the maximum specified AS.

The rapid progress observed in the field of neonatology in the last decades, the expanding knowledge base, and technological developments have resulted in increased survival of newborns. This has led to a significantly greater number of medical procedures undertaken immediately after birth. These procedures and their outputs should not be omitted in the health assessment of newborns [13, 15, 16]. Our results confirm the link between the interventions undertaken immediately after birth and the health status later in life. In 2006, the AAP Committee on Foetuses and Newborns and the ACOG Committee on Obstetric Practice proposed an expanded AS to account for these interventions and their effects [2, 16]. This scale contains, along with the classical AS, a listed course of resuscitation procedures. In use since 2008 in the Neonatal and Intensive Care Department of the Medical University of Warsaw, the expanded AS has significantly improved the precision of the documentation of the resuscitation procedures. It has also decreased the subjectivity of the health status evaluation of newborns. Therefore, this score was used in our study.

Further developments in the health assessment of newborns include the combined AS developed by Rudiger and Aguar [14], which merges the specified and expanded AS and assigns numeric values to the descriptive components of the expanded AS. Dalili et al. [17] compared the four types of AS and concluded that only a low score according to the combined AS predicts the incidence of IVH and its neurological complications in infants with perinatal hypoxia. A low combined AS at 5 minutes after birth has been independently linked to IVH incidence. However, it does not indicate the severity of IVH. These results agree with the ACOG and AAP, our results, and other published studies showing the low prognostic value of the classic AS on prospective neurological status or unfavourable health prognosis [18–23].

Our study was based on decision trees, which have several advantages over other decision algorithms. Their simple structure allows for an easy interpretation of the results. They enable the assessment of the importance of the variables and attributes and do not require assumptions regarding the specific distribution of the variables. The missing data do not cause problems in the analysis, and the classification based on decision trees is characterised by high accuracy. Additionally, the possibility of modelling dependencies of nonlinear phenomena and of analysing various sets of variables (nominal, ordinal, and continuous) allows for greater precision and detail in the description of the infants' health status and the undertaken interventions. The decision tree algorithm suited our study goals and setup because we required i) a simple data representation structure, ii) the possibility to analyse various sets of variables (nominal, ordinal, and continuous) with non-normal distribution, and iii) a high-accuracy classification system.

Our CHAID-based analysis shows the importance of the concentration of the oxygen supplied after birth in the prognosis of IVH III/IPH incidence in premature infants. In score-only-based systems this information is lost. The combined AS only considers the presence or absence of oxygen supply. In our analysis, we defined the thresholds for the selection of the groups of newborns for whom the health status prognosis can be made. Nevertheless, this is only a preliminary study that focuses on the prognosis of IVH III and IPH incidence based on an infant's health status after birth. The GA and the birth body weight are the most important risk factors for complications such as IVH, ROP, and BPD. Although these diseases have complex pathogenesis, the states of blood supply and oxygenation of cells and tissues are the key influencing factors [24, 25].

CONCLUSIONS

The concentration of oxygen used during resuscitation is an important factor in the prognosis of IVH III and IPH incidence. In follow-up studies, we plan to define the elements of the expanded AS that facilitate predictions of other early complications in preemies, like ROP and BPD. This information will not only improve the assessment of a newborn's health status but also allow doctors in labour wards to choose the most appropriate and effective interventions.

Acknowledgements

The authors would like to thank Proper Medical Writing Sp. z o.o. for providing medical writing assistance and editorial support in the development of this manuscript.

Conflict of interests

The authors declare no conflict of interest.

REFERENCES

1. Committee on Obstetric Practice American Academy of Pediatrics — Committee on Fetus and Newborn. Committee Opinion No. 644. The Apgar Score. Obstetrics & Gynecology. 2015; 126(4): e52–e55, doi: 10.1097/aog.0000000000001108.
2. American Academy of Pediatrics, Committee on Fetus and Newborn, American College of Obstetricians and Gynecologists and Committee on Obstetric Practice. The Apgar score. Pediatrics. 2006; 117(4): 1444–1447, doi: 10.1542/peds.2006-0325, indexed in Pubmed: 16585348.
3. Montgomery KS. Apgar Scores: Examining the Long-term Significance. J Perinat Educ. 2000; 9(3): 5–9, doi: 10.1624/105812400X87716, indexed in Pubmed: 17273212.
4. Hogan L, Ingemarsson I, Thorngren-Jerneck K, et al. How often is a low 5-min Apgar score in term newborns due to asphyxia? Eur J Obstet Gynecol Reprod Biol. 2007; 130(2): 169–175, doi: 10.1016/j.ejogrb.2006.03.002, indexed in Pubmed: 16621222.
5. Lee HC, Subeh M, Gould JB. Low Apgar score and mortality in extremely preterm neonates born in the United States. Acta Paediatr. 2010; 99(12): 1785–1789, doi: 10.1111/j.1651-2227.2010.01935.x, indexed in Pubmed: 20626363.
6. Jain L, Ferre C, Vidyasagar D, et al. Cardiopulmonary resuscitation of apparently stillborn infants: survival and long-term outcome. J Pediatr. 1991; 118(5): 778–782, doi: 10.1016/s0022-3476(05)80046-0, indexed in Pubmed: 2019934.
7. American College of Obstetrics and Gynecology. Neonatal Encephalopathy and Cerebral Palsy: Defining the Pathogenesis and Pathophysiology. Washington, DC ACOG. 2003.
8. Carter BM, Holditch-Davis D. Risk factors for necrotizing enterocolitis in preterm infants: how race, gender, and health status contribute. Adv Neonatal Care. 2008; 8(5): 285–290, doi: 10.1097/01.ANC.0000338019.56405.29, indexed in Pubmed: 18827518.
9. Chua CO, Vinukonda G, Hu F, et al. Effect of hyperoxic resuscitation on propensity of germinal matrix haemorrhage and cerebral injury. Neuropathol Appl Neurobiol. 2010; 36(5): 448–458, doi: 10.1111/j.1365-2990.2010.01087.x, indexed in Pubmed: 20408959.
10. Sánchez-Torres AM, García-Alix A, Cabañas F, et al. Impacto de la reanimación cardiopulmonar avanzada en recién nacidos pretérmino de extremado bajo peso [Impact of cardiopulmonary resuscitation on extremely low birth weight infants]. An Pediatr (Barc). 2007; 66: 38–44.
11. Szpecht D, Frydryszak D, Miszczyk N, et al. The incidence of severe intraventricular hemorrhage based on retrospective analysis of 35939 full-term newborns-report of two cases and review of literature. Childs Nerv Syst. 2016; 32(12): 2447–2451, doi: 10.1007/s00381-016-3164-5, indexed in Pubmed: 27392444.
12. Bokiniec R. Odległe skutki krwawień do ośrodkowego układu nerwowego u noworodków Pediatr Dypl. 2013; 17: 2–6.
13. Rüdiger M, Braun N, Aranda J, et al. TEST-Apgar Study-Group. Neonatal assessment in the delivery room--Trial to Evaluate a Specified Type of Apgar (TEST-Apgar). BMC Pediatr. 2015; 15: 18, doi: 10.1186/s12887-015-0334-7, indexed in Pubmed: 25884954.
14. Rüdiger M, Aguar M. Newborn Assessment in the Delivery Room. NeoReviews. 2012; 13(6): e336–e342, doi: 10.1542/neo.13-6-e336.
15. Dalili H, Nili F, Sheikh M, et al. Comparison of the four proposed Apgar scoring systems in the assessment of birth asphyxia and adverse early neurologic outcomes. PLoS One. 2015; 10(3): e0122116, doi: 10.1371/journal.pone.0122116, indexed in Pubmed: 25811904.
16. Committee on Obstetric Practice, ACOG, American Academy of Pediatrics; Committee on Fetus and Newborn, ACOG. ACOG Committee Opinion. Number 333, May 2006 (replaces No. 174, July 1996): The Apgar score. Obstet Gynecol. 2006; 107(5): 1209–1212, doi: 10.1097/00006250-200605000-00051, indexed in Pubmed: 16648434.
17. Dalili H, Sheikh M, Hardani AK, et al. Comparison of the Combined versus Conventional Apgar Scores in Predicting Adverse Neonatal Outcomes. PLoS One. 2016; 11(2): e0149464, doi: 10.1371/journal.pone.0149464, indexed in Pubmed: 26871908.
18. Fahey J, King TL. Intrauterine asphyxia: clinical implications for providers of intrapartum care. J Midwifery Womens Health. 2005; 50(6): 498–506, doi: 10.1016/j.jmwh.2005.08.007, indexed in Pubmed: 16260364.
19. Is the Apgar score outmoded? Lancet. 1989; 1(8638): 591–592, indexed in Pubmed: 2564115.
20. Marlow N. Do we need an Apgar score? Arch Dis Child. 1992; 67(7 Spec No): 765–767, doi: 10.1136/adc.67.7_spec_no.765, indexed in Pubmed: 1519971.
21. Behnke M, Eyler FD, Carter RL, et al. Predictive value of Apgar scores for developmental outcome in premature infants. Am J Perinatol. 1989; 6(1): 18–21, doi: 10.1055/s-2007-999536, indexed in Pubmed: 2462884.
22. Socol ML, Garcia PM, Riter S. Depressed Apgar scores, acid-base status, and neurologic outcome. Am J Obstet Gynecol. 1994; 170(4): 990–998, doi: 10.1016/s0002-9378(94)70092-3.
23. Padayachee NBD. Outcomes of neonates with perinatal asphyxia at a tertiary academic hospital in Johannesburg, South Africa. South African Journal of Child Health. 2013; 7: 89–94.
24. Shah P, Riphagen S, Beyene J, et al. Multiorgan dysfunction in infants with post-asphyxial hypoxic-ischaemic encephalopathy. Arch Dis Child Fetal Neonatal Ed. 2004; 89(2): F152–F155, doi: 10.1136/adc.2002.023093, indexed in Pubmed: 14977901.
25. Shah PS, Perlman M. Time courses of intrapartum asphyxia: neonatal characteristics and outcomes. Am J Perinatol. 2009; 26(1): 39–44, doi: 10.1055/s-0028-1095185, indexed in Pubmed: 18979407.

VIA MEDICA

REVIEW PAPER / GYNECOLOGY

Ginekologia Polska
2023, vol. 94, no. 2, 152–157

ISSN 0017–0011, e-ISSN 2543–6767

DOI 10.5603/GP.a2022.0147

Chronic endometritis — is it time to clarify diagnostic criteria?

Katarzyna Klimaszyk[1], Henriette Svarre Nielsen[2], Ewa Wender-Ozegowska[1], Malgorzata Kedzia[1]

[1]Department of Reproduction, Poznan University of Medical Sciences, Poznan, Poland
[2]The Recurrent Pregnancy Loss Unit, The Capital Region, The Fertility Clinic, Copenhagen University Hospitals Rigshospitalet and Hvidovre, Copenhagen, Denmark

ABSTRACT

Chronic endometritis is a persistent, low-intensity inflammation of endometrial mucosa, characterized by the infiltration of plasma cells into the endometrial stroma This immunological alteration is thought to be a consequence of a bacterial infection. For a long time, chronic endometritis was poorly investigated and rarely considered in clinical practice because it is either asymptomatic or presents with no specific symptoms. Its association with adverse effects on fertility and retrospectively reported effectiveness of antibiotic treatment were the main reasons for a growing interest in this endometrial pathology. Chronic endometritis is now a hot topic in recurrent pregnancy loss and recurrent implantation failure research.

Nevertheless, there are still no recommendations to include chronic endometritis investigation in a clinical evaluation of infertile patients. The uncertain role of this condition is an effect of significant differences in study results presented by different research groups. One important reason for these inconsistent findings is a lack of standardised chronic endometritis diagnostic methods.

We present a review of the literature, focusing on the currently available chronic endometritis diagnostic techniques. The review is subdivided into three parts concerning the diagnostic accuracy of three main diagnostic modalities. Histopathological examination of endometrial tissue, hysteroscopic evaluation of uterine cavity and identification of the bacterial factor.

In conclusion, it is of great importance to establish a consensus on the diagnostic criteria for chronic endometritis. This is the only way to enhance international cooperation and create well-design multicenter studies to evidence the role of this endometrial pathology in infertility.

Key words: chronic endometritis; diagnostic techniques; immunohistochemistry; hysteroscopy; endometrial microbiome

Ginekologia Polska 2023; 94, 2: 152–157

INTRODUCTION

The past few years have seen a notable increase in the number of publications focused on chronic endometritis. This increase is associated with a growing interest in understanding the endometrial factors in infertility and their roles in the implantation process.

Chronic endometritis (CE) is a persistent but low-intensity inflammation of the endometrium, mostly asymptomatic or correlated with non-specific symptoms, namely pelvic pain, dysfunctional uterine bleeding, and vaginal discharge [1]. Nevertheless greater interest in this long-known endometrium pathology is associated with the appearance of data showing a correlation between CE and adverse reproduction outcomes [2–4].

Classic methods used in the CE investigation process include microbial culture, hysteroscopy, and histopathology examination of endometrial samples. As the diagnostic gold standard serves histopathological identification of plasma cells in the endometrial biopsy [5, 6].

The first step in the clinical concern regarding CE was proving the higher than previously believed prevalence of this pathology [3]. The immunohistochemistry staining

Corresponding author:
Katarzyna Klimaszyk
Department of Reproduction, Poznan University of Medical Sciences, 33 Polna St, 60–535 Poznan, Poland
phone: +48 889046505
e-mail: kedjaworska@gmail.com

Received: 8.11.2022 Accepted: 11.11.2022 Early publication date: 9.12.2022

used in the histopathological evaluation of endometrial tissue demonstrated that CE is underdiagnosed and in fact more common in patients with recurrent pregnancy losses [3]. This finding gave a basis for further investigation into CE effects on fertility.

The reported effectiveness of antibiotic treatment in CE resolution, confirmed in repeated histological examination of endometrial tissue, was another milestone in building the significance of this diagnosis [2, 7–11]. Some publications showed improvement in reproductive outcomes of patients after effective antibiotic treatment of CE [10, 12]. These reports caused even greater interest in CE investigation, as there is great value in finding potentially treatable causes of fertility alternation. Despite all the promising reports, CE investigation is not recommended in guidelines for the clinical management of patients with infertility [13, 14]. The main reason is the lack of sufficient evidence from prospective observational studies and randomized controlled trials on the predictive value of a positive test for CE. Performing a meta-analysis of the available data is biased by the significant heterogeneity of the CE diagnostic criteria used by different researchers [15, 16].

The CE estimated rate in the general population is hard to define due to the lack of a characteristic clinical manifestation. The reported range in the population of infertile patients varies between 2.8% and 39.0%, while in a selected group of women diagnosed with unexplained recurrent miscarriage or repeated implantation failure it may be as high as 60% or 66%, respectively [2, 6, 9, 17, 18].

This wide range of reported CE prevalence is a consequence of the fact that its diagnosis depends on the method of detection. As shown in a study by Moreno, when the three classic diagnostic techniques are applied to the same patients it may yield contradictory results [19].

In this paper, we aim to review the literature regarding diagnostic techniques used in CE investigation.

MATERIAL AND METHODS

We studied the diagnostic techniques and diagnostic criteria used by researchers investigating chronic endometritis. A search of PubMed and Embase was performed to identify relevant studies, with a restriction to English language articles. The following keywords and their combinations were used: "chronic endometritis", "infertility", and "diagnostic criteria". Additional searches included references from identified publications.

Diagnostic techniques

Histopathology

The histological detection of plasma cells in endometrial tissue is a generally accepted gold standard CE diagnostic method [5, 6]. Although plasma cell identification in endometrium specimens stained with hematoxylin and eosin (H&E) is possible, it can be challenging, time-consuming and subjective. Therefore immunohistochemistry (IHC) has been introduced to detect plasma cell marker syndecan-1 (CD 138).

Syndecan-1 (CD138) is a transmembrane heparan sulfate proteoglycan, involved in cell-cell and cell-matrix adhesion. The expression of syndecan-1 is typically observed on the cell membrane of plasma cells and mature epithelial cells [20]. This type of plasma cell identification has been successfully used in diagnosing plasma cell tumors, including multiple myelomas [21].

One advantage of IHC CD138 staining is the ability to identify not only typical round plasma cells with classic features of clock-face chromatin in an eccentrically placed nucleus with a perinuclear halo but also untypical spindle-shaped ones [22]. This is important, because abundant stromal mitoses and stromal cell proliferation in CE may mask the characteristic features of the plasma cells and increase the chance of them being overlooked by a pathologist.

Moreover, IHC CD138 is found to be an objective plasma cell identification method and can decrease the number of false-positive results. It reduces the chance of counting other cells by mistake, such as mononuclear and plasmacytoid stromal cells instead of plasma cells and increases intra-observer inter-observer agreement in the diagnosis [23].

Studies by McQueen show that the use of IHC CD138 staining significantly increased the number of plasma cells detected in endometrium samples of women with recurrent pregnancy loss. This confirms the increased sensitivity of IHC CD 138 plasma cell identification compared to H&E staining [3, 24].

Lately, two research groups introduced the multiple myeloma 1 (MUM1) protein as a plasma cell marker in the chronic endometritis study [25, 26]. MUM1 also known as interferon regulatory factor 4 (IRF4) is a transcription factor protein expressed in plasma cells, activated B and T cells. MUM1 IHC staining pattern is primarily nuclear and overcomes the disadvantage of background reaction present in the CD138 staining.

Due to the greater sensitivity of plasma cell detection, researchers now must answer the question: how many plasma cells per tissue sample area is enough for the diagnosis of CE?

In examining recently published original articles, we find a huge variety in the applied histopathological diagnostic criteria. Many investigators use different methods of quantification, while the threshold number of plasma cells per tissue sample can be set between strict and broad [15].

The results obtained from studies designed with such heterogeneity in the basic diagnostic criteria range vary

significantly, causing bias in metanalysis and comparative analysis [15].

Few research groups have chosen to analyse this problem and compare the prevalence of CE determined by means of different histopathological criteria, to evaluate the most accurate one [16, 27, 28].

Hirata et al. [27] analysed four threshold numbers of plasma cells used in the same group of patients undergoing in vitro fertilisation (IVF) procedures. Based on the comparison of differences in pregnancy rate, live birth rates and miscarriage rate among CE and non-CE groups defined by four different criteria, they concluded that CE should be defined as the presence of ≥ 1 plasma cell per 10 high-power fields (HPF) [27].

Another approach to this problem was demonstrated by Y. Liu et al. in their study [16]. They set the reference range of plasma cells derived from the examination of endometrium tissue samples from a control group of 40 fertile patients. In the study group of females with recurrent pregnancy loss (RPL) and recurrent implantation failure (RIF), they considered plasma cell numbers above the 95th percentile of the reference value as indicative of CE diagnosis. The threshold level for three different methods of quantification were: 1.95 CD138 plasma cells per ten randomly chosen HPFs, 2.95 CD138 plasma cells per section, and 5.15 CD138 plasma cells per 0.1 mm^2 [16].

To tackle the problem of redefining chronic endometritis, McQueen et al. [24] carried out a study to compare the prevalence of CE among women with RPL and the control group, using various histopathological definitions. The novelty of the concept was to include endometrial stromal changes defined as the spindling of cells, oedema, breakdown pigment deposition, areas of hypercellularity and the presence of inflammatory cells other than plasma cells. The authors achieved the highest diagnostic sensitivity and specificity when CE was defined as the presence of one or more plasma cells per 10 HPFs in the setting of endometrial stromal changes.

The lack of worldwide consensus on histopathological criteria of CE is demonstrated in the results of the survey of pathologists, asking about diagnostic criteria they follow in clinical practice [29]. This study shows that we need clarification of histopathological CE criteria, especially as it is a verification method in the search for other CE diagnostic modalities.

Hysteroscopy

Hysteroscopy assessment of uterine anatomy is one of the recommended procedures in the diagnostic process of abnormal uterine bleeding, RPL, infertility, and suspected intrauterine lesions [14]. The possibility to identify changes in endometrium appearance caused by persistent inflammation led to a number of studies investigating this CE diagnostic modality.

Visual signs suggesting CE, described in the literature are micro polyps, focal hyperaemia, stromal oedema, and endometrial strawberry aspect, defined as large areas of hyperaemic endometrium flushed with white central points [30–32]. Nevertheless, the exact diagnostic criteria and reliability of this method remain a subject of debate [5, 30–35].

The most common method used to evaluate the diagnostic accuracy of hysteroscopic findings is to perform a hysteroscopic examination with subsequent endometrial biopsy and histopathological verification of the CE diagnoses.

In most studies, a hysteroscopic examination was performed in the proliferative phase of the endometrium cycle. Reported sensitivity, specificity, and the positive and negative predictive values differed depending on which set of visual features the diagnosis was based on. For example, in the study by Cicinelli et al., when detection of oedema and hyperaemia was set as a criterium of CE, 92% sensitivity, 93% specificity, 64% positive and 99% negative predictive values were reported [31, 32]. However, when the presence of micro polyps was also included, the specificity increased to 99.0% while the sensitivity decreased to 55.4% [31]. The authors concluded that the absence of endometrial hyperaemia and oedema was sufficient to rule out chronic endometritis, while the presence of micro polyps was a very reliable visual feature, although not very common in CE patients. It is worth noting that these studies can be biased because no ICH staining was used in the histopathological verification process [32].

The next research group aiming to evaluate the role of hysteroscopy also included the same three hysteroscopic features suggestive of CE in a large cohort of 1189 patients [5]. An advantage of this study was the fact that the verification method was a histopathological examination with the use of IHC for CD138 plasma cell identification. The reported sensitivity of a hysteroscopic diagnosis based on the presence of at least one of three features was only 59.3% and a specificity of 69.7%. The specificity increased to 99% when at least two features were found simultaneously in the same patient, while the sensitivity dropped to 5% [5]. The conclusion from that study was that the presence of the hysteroscopic features of CE should lead to a diagnosis, increasing the likelihood of histological confirmation, but the lack of alarming features cannot rule out the diagnosis. The authors highlighted that hysteroscopy should not replace histopathological examination as a CE diagnostic method of choice. Another interesting aspect analysed in Dongmei Song's study was the correlation between the number of plasma cell counts and the hysteroscopic findings. The study showed that the higher the rate of plasma

cells per 10 HPF, the more likely occurrence of the hysteroscopic features of CE was [5].

The need to develop a diagnostic consensus emerged from the variety of hysteroscopy diagnostic accuracy reported by different research groups. In 2019, the 'Working Group for Standardization of Chronic Endometritis Diagnosis' reached a consensus with the use of the Delphic method. Experts established diagnostic criteria which included the presence of at least one of the following hysteroscopic findings: strawberry aspect, focal hyperaemia, haemorrhagic spots, micro polyps, and stromal oedema in the follicular phase [36]. The major disadvantage of hysteroscopic examination is the fact that visual assessment of the uterine cavity is subjective and may depend upon the physician`s experience. That is why the reproducibility of newly established diagnostic criteria was evaluated. According to an international randomized-controlled observer study, knowledge of unified criteria increases physicians' ability to detect and diagnose cases of CE without increasing false-positive diagnoses [36].

In a systemic review aimed at answering whether hysteroscopy was suitable for setting the CE diagnosis, the authors did not manage to support the hypothesis. They included 15 studies with a total of 5526 participants, but due to the heterogeneity of the diagnostic criteria used in those studies, the data was not sufficient to confirm that hysteroscopy alone was not adequate for setting the diagnosis [37].

It is worth emphasising that the lack of standardized histopathological CE criteria results in a lack of a concise verification method of hysteroscopic findings in various studies.

Identification of the bacterial factor

Microbial infection is believed to be the primary cause of persistent inflammation of the endometrial lining present in chronic endometritis [33, 38]. The main finding supporting the theory of the infectious genesis of CE is the effectiveness of antibiotic treatment on the histopathologically confirmed resolution of this endometrial pathology shown in a prospective randomized control trial [7].

The classic technique of bacterial identification used in CE investigations is a microbial culture of the endometrial tissue. It is worth noting that the study by Cicinelli et al. [39] showed a low concordance of vaginal and endocervical bacterial findings with those from endometrium sampling. These findings implicate that samples obtained from the lower genital tract cannot be used in the CE diagnostic process.

The main advantage of microbial culture is the objective identification of the endometrial pathogens and the possibility of administering a targeted antibiogram-guided treatment.

Findings from the Cicineli and Kitaya research groups show us that the pathogens detected by microbial culture in patients with a CE diagnosis were mostly the common bacteria *Streptococcus species*, *Escherichia coli*, *Enterococcus faecalis* and *Staphylococcus species*, *Mycoplasma*/*Ureaplasma species*, *Proteus species*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Gardnerella vaginalis*, *Corynebacterium species* and yeast [33, 39, 40].

The major limitation of this diagnostic technique is the fact that not all bacteria are culturable under standard laboratory conditions. The reported rate of positive microbial culture in histopathologically confirmed CE cases varies between 52% and 73% [19, 33].

It is well proven that the uterine cavity is not sterile under normal physiological conditions [41–44]. This is why negative bacterial culture results are most probably a result of method limitations, rather than the actual lack of bacteria in the uterine cavity. The use of molecular techniques enables the detection of low biomass uterine microbiota. These techniques include the quantitive polymerase chain reaction and next-generation sequencing of the 16S RNA bacteria. Therefore, the modern concept behind the CE pathophysiological model focuses more on microbial and immune cross-talk rather than the presence of bacteria in the uterine cavity itself [45].

The role of uterine microbiota and its influence on the decidualization and receptivity of the endometrium in infertile patients is now wildly investigated.

According to the results of a prospective pilot study by Moreno et al [43] bacterial DNA was detected in all of the endometrial fluid samples examined using PCR and 16S RNA sequencing. In a larger group of patients detectable amount of DNA was found in 61% of endometrial fluid samples and 64% of endometrial biopsy samples [44].

Based on the uterine microbiota composition, *Lactobacillus*-dominated microbiota (> 90% *Lactobacillus spp.*) or a non-*Lactobacillus*-dominated microbiota (< 90% *Lactobacillus spp.* with > 10% of other bacteria) was defined [43]. Reported reproductive outcomes of patients with non-*Lactobacillus*-dominated endometrial microbiota undergoing IVF procedures were significantly worse compared to a group with *Lactobacillus*-dominated endometrial microbiota. For example, the implantation rate was 60.7% vs 23.1% while the live birth rate was 58.8% vs 6.7% respectively [43]. Unfortunately, this study did not include a histopathological assessment of CE and its correlation with microbial findings.

The study conducted by Moreno in collaboration with Cicinelli was designed to evaluate the diagnostic accuracy of the molecular diagnostic tools used in a CE investigation. The histology, hysteroscopy and microbial culture results were compared with the RT-PCR identification of nine pathogens in 65 patients. These nine pathogens were

selected based on the findings of the most common bacteria in patients with histopathological confirmed CE. Based on the cases of the concordant finding of all three classic diagnostic techniques compared with RT-PCR findings, 75% sensitivity, 100% specificity and 77% accuracy of this molecular diagnostic tool were reported. This demonstrated an opportunity to overcome the bias of classic diagnostic methods and give new diagnostic tools in this infection pathology of endometrium [19]. It is worth indicating that in this study only 20% of 65 patients got unanimous results of all three classic techniques. Therefore, the vast majority encountered ambiguous results, showing that CE diagnosis determined by means of different diagnostic methods may yield contradictory results.

CONCLUSIONS

Despite accumulating reports on CE association with poor reproductive outcomes and the evidenced effectiveness of antibiotic treatment on CE resolution, this inflammatory condition is not routinely investigated in patients with infertility.

Clinical guidelines do not recommend CE investigation since more prospective observational studies and randomized controlled trials are needed.

The first step towards that is creating precise diagnostic criteria concerning CE for researchers all around the world to follow. Unified diagnostic criteria will lead to an opportunity to perform high-quality meta-analyses to gather results from the rising number of studies investigating this condition.

As was already highlighted in this review, the histopathological examination of endometrial samples is, for now, a diagnostic gold standard in CE. It is also the verification method used for assessing the diagnostic accuracy of other CE diagnostic techniques. Therefore, it is vital to reach an international consensus on universally accepted standardized histopathological criteria for CE. A precise threshold number of plasma cells identified including the use of ICH staining is needed.

With a unified histopathological verification method, further studies on the value of hysteroscopy and microbial identification of bacterial factors will give more precise results.

The use of molecular microbiology technology seems to be the future of understanding the role of the human microbiome in the aetiology of many medical conditions. Reproductive health is not an exception as new possibilities shed light on the relationship between endometrium bacterial community profiling and pregnancy outcomes. However, before implementing this diagnostic technique into clinical practice we need a verification method to access the relevance of these findings.

In conclusion, it is of great importance for the sociates of gynaecologists and pathologists to unify CE diagnostic criteria and create a clinical investigation scheme. This might be the way to reduce the inconsistency of CE study results and help to prove the significance of CE screening in infertile patients.

Acknowledgements

Katarzyna Klimaszyk is a participant of STER Internationalisation of Doctoral Schools Programme from NAWA Polish National Agency for Academic Exchange No. PPI/STE/2020/1/00014/DEC/02.

Conflict of interest

The authors declare that there are no competing interests in this study.

REFERENCES

1. Greenwood SM, Moran JJ. Chronic endometritis: morphologic and clinical observations. Obstet Gynecol. 1981; 58(2): 176–184, indexed in Pubmed: 7254729.
2. Cicinelli E, Matteo M, Tinelli R, et al. Chronic endometritis due to common bacteria is prevalent in women with recurrent miscarriage as confirmed by improved pregnancy outcome after antibiotic treatment. Reprod Sci. 2014; 21(5): 640–647, doi: 10.1177/1933719113508817, indexed in Pubmed: 24177713.
3. McQueen DB, Perfetto CO, Hazard FK, et al. Pregnancy outcomes in women with chronic endometritis and recurrent pregnancy loss. Fertil Steril. 2015; 104(4): 927–931, doi: 10.1016/j.fertnstert.2015.06.044, indexed in Pubmed: 26207958.
4. Wiesenfeld HC, Hillier SL, Meyn LA, et al. Subclinical pelvic inflammatory disease and infertility. Obstet Gynecol. 2012; 120(1): 37–43, doi: 10.1097/AOG.0b013e31825a6bc9, indexed in Pubmed: 22678036.
5. Song D, Li TC, Zhang Y, et al. Correlation between hysteroscopy findings and chronic endometritis. Fertil Steril. 2019; 111(4): 772–779, doi: 10.1016/j.fertnstert.2018.12.007, indexed in Pubmed: 30683588.
6. Kasius JC, Broekmans FJM, Sie-Go DM, et al. The reliability of the histological diagnosis of endometritis in asymptomatic IVF cases: a multicenter observer study. Hum Reprod. 2012; 27(1): 153–158, doi: 10.1093/humrep/der341, indexed in Pubmed: 22025228.
7. Song D, He Y, Wang Y, et al. Impact of antibiotic therapy on the rate of negative test results for chronic endometritis: a prospective randomized control trial. Fertil Steril. 2021; 115(6): 1549–1556, doi: 10.1016/j.fertnstert.2020.12.019, indexed in Pubmed: 33549312.
8. Cicinelli E, Resta L, Loizzi V, et al. Antibiotic therapy versus no treatment for chronic endometritis: a case-control study. Fertil Steril. 2021; 115(6): 1541–1548, doi: 10.1016/j.fertnstert.2021.01.018, indexed in Pubmed: 33722376.
9. Cicinelli E, Matteo M, Tinelli R, et al. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. Hum Reprod. 2015; 30(2): 323–330, doi: 10.1093/humrep/deu292, indexed in Pubmed: 25385744.
10. Xiong Y, Chen Qi, Chen C, et al. Impact of oral antibiotic treatment for chronic endometritis on pregnancy outcomes in the following frozen-thawed embryo transfer cycles of infertile women: a cohort study of 640 embryo transfer cycles. Fertil Steril. 2021; 116(2): 413–421, doi: 10.1016/j.fertnstert.2021.03.036, indexed in Pubmed: 33926717.
11. Gay C, Hamdaoui N, Pauly V, et al. Impact of antibiotic treatment for chronic endometritis on unexplained recurrent pregnancy loss. J Gynecol Obstet Hum Reprod. 2021; 50(5): 102034, doi: 10.1016/j.jogoh.2020.102034, indexed in Pubmed: 33307243.
12. Cicinelli E, Matteo M, Trojano G, et al. Chronic endometritis in patients with unexplained infertility: Prevalence and effects of antibiotic treatment on spontaneous conception. Am J Reprod Immunol. 2018; 79(1), doi: 10.1111/aji.12782, indexed in Pubmed: 29135053.
13. Practice Committee of the American Society for Reproductive Medicine. Electronic address: asrm@asrm.org, Practice Committee of the

American Society for Reproductive Medicine. Evidence-based treatments for couples with unexplained infertility: a guideline. Fertil Steril. 2020; 113(2): 305–322, doi: 10.1016/j.fertnstert.2019.10.014, indexed in Pubmed: 32106976.
14. Bender Atik R, Christiansen OB, Elson J, et al. ESHRE Guideline Group on RPL. ESHRE guideline: recurrent pregnancy loss. Hum Reprod Open. 2018; 2018(2): hoy004, doi: 10.1093/hropen/hoy004, indexed in Pubmed: 31486805.
15. Huang W, Liu Bo, He Y, et al. Variation of diagnostic criteria in women with chronic endometritis and its effect on reproductive outcomes: A systematic review and meta-analysis. J Reprod Immunol. 2020; 140: 103146, doi: 10.1016/j.jri.2020.103146, indexed in Pubmed: 32442825.
16. Liu Y, Chen X, Huang J, et al. Comparison of the prevalence of chronic endometritis as determined by means of different diagnostic methods in women with and without reproductive failure. Fertil Steril. 2018; 109(5): 832–839, doi: 10.1016/j.fertnstert.2018.01.022, indexed in Pubmed: 29778382.
17. Farooki MA. Epidemiology and pathology of chronic endometritis. Int Surg. 1967; 48(6): 566–573, indexed in Pubmed: 6064728.
18. Johnston-MacAnanny EB, Hartnett J, Engmann LL, et al. Chronic endometritis is a frequent finding in women with recurrent implantation failure after in vitro fertilization. Fertil Steril. 2010; 93(2): 437–441, doi: 10.1016/j.fertnstert.2008.12.131, indexed in Pubmed: 19217098.
19. Moreno I, Cicinelli E, Garcia-Grau I, et al. The diagnosis of chronic endometritis in infertile asymptomatic women: a comparative study of histology, microbial cultures, hysteroscopy, and molecular microbiology. Am J Obstet Gynecol. 2018; 218(6): 602.e1–602.e16, doi: 10.1016/j.ajog.2018.02.012, indexed in Pubmed: 29477653.
20. Inki P. Expression of syndecan-1 in female reproductive tract tissues and cultured keratinocytes. Mol Hum Reprod. 1997; 3(4): 299–305, doi: 10.1093/molehr/3.4.299 , indexed in Pubmed: 9237257.
21. O'Connell F, Pinkus J, Pinkus G. CD138 (Syndecan-1), a Plasma Cell Marker. Am J Clin Pathol. 2004; 121(2): 254–263, doi: 10.1309/617dwb-5gnfwxhw4l.
22. Kannar V, Lingaiah HK, Sunita V. Evaluation of endometrium for chronic endometritis by using syndecan-1 in abnormal uterine bleeding. J Lab Physicians. 2012; 4(2): 69–73, doi: 10.4103/0974-2727.105584, indexed in Pubmed: 23440678.
23. Kitaya K, Yasuo T. Inter-observer and intra-observer variability in immunohistochemical detection of endometrial stromal plasmacytes in chronic endometritis. Exp Ther Med. 2013; 5(2): 485–488, doi: 10.3892/etm.2012.824, indexed in Pubmed: 23407614.
24. McQueen DB, Maniar KP, Hutchinson A, et al. Redefining chronic endometritis: the importance of endometrial stromal changes. Fertil Steril. 2021; 116(3): 855–861, doi: 10.1016/j.fertnstert.2021.04.036, indexed in Pubmed: 34120737.
25. Cicinelli E, Haimovich S, De Ziegler D, et al. International Working Group for Standardization of Chronic Endometritis Diagnosis. MUM-1 immunohistochemistry has high accuracy and reliability in the diagnosis of chronic endometritis: a multi-centre comparative study with CD-138 immunostaining. J Assist Reprod Genet. 2022; 39(1): 219–226, doi: 10.1007/s10815-021-02356-1, indexed in Pubmed: 34791588.
26. Parks RN, Kim CJ, Al-Safi ZA, et al. Multiple myeloma 1 transcription factor is superior to CD138 as a marker of plasma cells in endometrium. Int J Surg Pathol. 2019; 27(4): 372–379, doi: 10.1177/1066896918814307, indexed in Pubmed: 30482071.
27. Hirata K, Kimura F, Nakamura A, et al. Histological diagnostic criterion for chronic endometritis based on the clinical outcome. BMC Womens Health. 2021; 21(1): 94, doi: 10.1186/s12905-021-01239-y, indexed in Pubmed: 33663485.
28. Li Y, Xu S, Yu S, et al. Diagnosis of chronic endometritis: How many CD138 cells/HPF in endometrial stroma affect pregnancy outcome of infertile women? Am J Reprod Immunol. 2021; 85(5): e13369, doi: 10.1111/aji.13369, indexed in Pubmed: 33152123.
29. Margulies SL, Dhingra I, Flores V, et al. The diagnostic criteria for chronic endometritis: a survey of pathologists. Int J Gynecol Pathol. 2021; 40(6): 556–562, doi: 10.1097/PGP.0000000000000737, indexed in Pubmed: 33323860.
30. Zolghadri J, Momtahan M, Aminian K, et al. The value of hysteroscopy in diagnosis of chronic endometritis in patients with unexplained recurrent spontaneous abortion. Eur J Obstet Gynecol Reprod Biol. 2011; 155(2): 217–220, doi: 10.1016/j.ejogrb.2010.12.010, indexed in Pubmed: 21232841.
31. Cicinelli E, Resta L, Nicoletti R, et al. Endometrial micropolyps at fluid hysteroscopy suggest the existence of chronic endometritis. Hum Reprod. 2005; 20(5): 1386–1389, doi: 10.1093/humrep/deh779, indexed in Pubmed: 15734762.
32. Cicinelli E, Resta L, Nicoletti R, et al. Detection of chronic endometritis at fluid hysteroscopy. J Minim Invasive Gynecol. 2005; 12(6): 514–518, doi: 10.1016/j.jmig.2005.07.394, indexed in Pubmed: 16337579.
33. Cicinelli E, De Ziegler D, Nicoletti R, et al. Chronic endometritis: correlation among hysteroscopic, histologic, and bacteriologic findings in a prospective trial with 2190 consecutive office hysteroscopies. Fertil Steril. 2008; 89(3): 677–684, doi: 10.1016/j.fertnstert.2007.03.074, indexed in Pubmed: 17531993.
34. Zargar M, Ghafourian M, Nikbakht R, et al. Evaluating chronic endometritis in women with recurrent implantation failure and recurrent pregnancy loss by hysteroscopy and immunohistochemistry. J Minim Invasive Gynecol. 2020; 27(1): 116–121, doi: 10.1016/j.jmig.2019.02.016, indexed in Pubmed: 30851430.
35. Bouet PE, El Hachem H, Monceau E, et al. Chronic endometritis in women with recurrent pregnancy loss and recurrent implantation failure: prevalence and role of office hysteroscopy and immunohistochemistry in diagnosis. Fertil Steril. 2016; 105(1): 106–110, doi: 10.1016/j.fertnstert.2015.09.025, indexed in Pubmed: 26456229.
36. Cicinelli E, Vitagliano A, Kumar A, et al. International Working Group for Standardization of Chronic Endometritis Diagnosis. Unified diagnostic criteria for chronic endometritis at fluid hysteroscopy: proposal and reliability evaluation through an international randomized-controlled observer study. Fertil Steril. 2019; 112(1): 162–173.e2, doi: 10.1016/j.fertnstert.2019.03.004, indexed in Pubmed: 31104760.
37. Gkrozou F, Tsonis O, Dimitriou E, et al. In women with chronic or subclinical endometritis is hysteroscopy suitable for setting the diagnosis? A systematic review. J Obstet Gynaecol Res. 2020; 46(9): 1639–1650, doi: 10.1111/jog.14360, indexed in Pubmed: 32578286.
38. Buzzaccarini G, Vitagliano A, Andrisani A, et al. Chronic endometritis and altered embryo implantation: a unified pathophysiological theory from a literature systematic review. J Assist Reprod Genet. 2020; 37(12): 2897–2911, doi: 10.1007/s10815-020-01955-8, indexed in Pubmed: 33025403.
39. Cicinelli E, De Ziegler D, Nicoletti R, et al. Poor reliability of vaginal and endocervical cultures for evaluating microbiology of endometrial cavity in women with chronic endometritis. Gynecol Obstet Invest. 2009; 68(2): 108–115, doi: 10.1159/000223819, indexed in Pubmed: 19521097.
40. Kitaya K, Matsubayashi H, Takaya Y, et al. Live birth rate following oral antibiotic treatment for chronic endometritis in infertile women with repeated implantation failure. Am J Reprod Immunol. 2017; 78(5), doi: 10.1111/aji.12719, indexed in Pubmed: 28608596.
41. Chen C, Song X, Wei W, et al. The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. Nat Commun. 2017; 8(1): 875, doi: 10.1038/s41467-017-00901-0, indexed in Pubmed: 29042534.
42. Ding T, Schloss PD. Dynamics and associations of microbial community types across the human body. Nature. 2014; 509(7500): 357–360, doi: 10.1038/nature13178, indexed in Pubmed: 24739969.
43. Moreno I, Codoñer FM, Vilella F, et al. Evidence that the endometrial microbiota has an effect on implantation success or failure. Am J Obstet Gynecol. 2016; 215(6): 684–703, doi: 10.1016/j.ajog.2016.09.075, indexed in Pubmed: 27717732.
44. Moreno I, Garcia-Grau I, Perez-Villaroya D, et al. Endometrial microbiota composition is associated with reproductive outcome in infertile patients. Microbiome. 2022; 10(1): 1, doi: 10.1186/s40168-021-01184-w, indexed in Pubmed: 34980280.
45. D'Ippolito S, Di Nicuolo F, Pontecorvi A, et al. Endometrial microbes and microbiome: Recent insights on the inflammatory and immune "players" of the human endometrium. Am J Reprod Immunol. 2018; 80(6): e13065, doi: 10.1111/aji.13065, indexed in Pubmed: 30375712.

VIA MEDICA

REVIEW PAPER / OBSTETRICS

Ginekologia Polska
2023, vol. 94, no. 2, 158–166

ISSN 0017–0011, e-ISSN 2543–6767

DOI 10.5603/GP.a2022.0148

Influence of selected factors on serum AFP levels in pregnant women in terms of prenatal screening accuracy — literature review

Joanna Glowska-Ciemny[1], Marcin Szymanski[1], Jakub Pankiewicz[1], Zbyszko Malewski[1, 2], Constantin von Kaisenberg[3], Rafal Kocylowski[1]

[1]PreMediCare New Med Medical Center, Poznan, Poland
[2]Division of Perinatology and Women's Diseases, Poznan University of Medical Sciences, Poznan, Poland
[3]Department of Obstetrics and Gynecology, Hannover Medical School, Germany

ABSTRACT

Alpha-fetoprotein (AFP) is one of the biochemical components of the triple (T-3) and quadruple (T-4) test used so far in prenatal screening mainly for trisomy 21 (T21) and neural tube defects (NTDs). Based on many years of experience and data collected during these studies, a variety of factors have been identified that can affect a pregnant woman's serum AFP level, and thus the risk assessment of trisomy 21 (T21) and neural tube defects. These include both unaccounted for purely medical data (e.g., from baseline information about the patient, assisted reproduction methods used, comorbidities and emerging pregnancy pathologies) and errors made during statistical analysis. Since the triple or quadruple test is usually performed between 15 and 20 weeks of pregnancy, most scientific studies are based solely on results from this period of pregnancy — limited data are available for the first and third trimesters of pregnancy. In the era of new improved screening tests, AFP has the potential to become an independent marker for pregnancy well-being evaluation.

Key words: prenatal screening; false positive/negative; triple/quadruple test; alpha-fetoprotein; AFP MoM; trisomy 21; neural tube defects

Ginekologia Polska 2023; 94, 2: 158–166

INTRODUCTION

In use since the late 1980s and early 1990s, the triple test [alpha-fetoprotein (AFP), human beta chorionic gonadotropin beta-hCG, estriol E3] and then the quadruple test (AFP, beta-hCG, E3, inhibin A) was a milestone in the biochemical diagnosis of aneuploidy. However, despite the undeniable advantages demonstrated by years of use, it is burdened with certain drawbacks. In the era of the spread of fetal deoxyribonucleic acid (DNA), the sensitivity of the triple test at about 67–73% and quadruple test at 75–85% (depending on the cutoff point for false positive rate, FPR) is no longer particularly awe-inspiring. Another problem is the 4–8% false-positive rate for trisomy 21 (as high as 21–25% after an age of 35), and 5–14% for the quadruple test, causing concern in patients whose first trimester composite test result [according to Fetal Medicine Foundation (FMF)] and second trimester fetal anatomy evaluation were normal [1–3]. In addition, like any laboratory test, this test is susceptible to the influence of individual factors specific to the patient, her comorbidities, diagnostic and statistical errors. This review paper aims to systematize and characterize the factors that directly influence AFP (and AFP multiple of the median — MoM) levels, and ultimately the incidence of false-positive and negative prenatal screening tests based on its evaluation (Tab. 1 and 2).

The available data suggest the possibility of using AFP as a separate marker of pregnancy well-being indicating pregnancy abnormalities other than those already present after considering all known distractors of its serum levels in pregnant women [4].

BASELINE INTERVIEW

Race

Black and yellow race patients have higher AFP levels than white Caucasians. The difference in AFP levels between

Corresponding author:
Joanna Glowska-Ciemny
PreMediCare New Med Medical Center, 21 Czarna Rola St, 61–625 Poznan, Poland
e-mail: jglowskaciemy@gmail.com

Received: 22.08.2022 Accepted: 21.11.2022 Early publication date: 29.12.2022

Table 1. Pregnancy periods during which the effects of selected factors on AFP levels were studied

Factor	I trimester	II trimester	III trimester
Mother race	√	√	√
Mother BMI	√	√	√
Parity	√	√	√
Pregnancy age	√	√	√
Cigarettes	√	√	√
Alcohol		√	
Fetus gender		√	
DM 1 and DM 2	√	√	
SLE		√	
Thrombophilia		√	
Hypertension		√	
OSA		√	
GDM		√	
PIH/PE	√	√	√
ICP		√	
Hyperemesis gravidarum		√	
Hemoglobin level		√	
Serological conflict		√	
IVF	√	√	
IVF-ICSI		√	
IVF — frozen embryos		√	
IVF — oocytes donation		√	
Vanishing twin	√	√	
CVS	√		
Subchorionic hematoma	√		
PMD		√	
PAS		√	
Circumvallate placenta		√	
Uterine myomas		√	
HIV+		√	
HSV2+ (fetal infection)		√	
Parvovirus B19		√	
Antiepileptic immunosuppressants, used to treat HIV+, inducing ovulation		√	
Folic acid deficiency		√	

BMI — body mass index; CVS — chorionic villus sampling; DM — diabetes mellitus; GDM — gestational diabetes mellitus; HIV — human immunodeficiency viruses; HSV2 — herpes simplex virus type 2; ICP — intrahepatic cholestasis of pregnancy; IVF — *in vitro* fertilization; PAS — placenta accreta spectrum; PIH/PE — pregnancy-induced hypertension/preeclampsia; PMD — placental mesenchymal dysplasia; OSA — obstructive sleep apnea; SLE — systemic lupus erythematosus

Table 2. Factors affecting maternal serum AFP levels (MS-AFP)

↑ higher AFP values	↔ no effect on AFP level	↓ lower AFP values
Male fetuses	Hyperemesis gravidarum	Female fetuses
Vanishing twin	Uterine myomas	Multiparity#
Clack and yellow race #	OSA	Obesity#
Alcohol	ICP	DM 1 and 2#
Cigarettes#	Weight of a newborn from a previous birth	GDM
SLE	Hypertension	Maternal anemia
PE	End-stage renal failure	Maternal polycythemia
Serological conflict	PIH	Folic acid supplementation
Parvovirus B19		HIV
HSV-2		Protease inhibitors
CVS		IVF#
Subchorionic hematoma		IVF-ICSI
PMD		
PAS		
Circumvallate placenta		
Antiphospholipid syndrome		
IVF — frozen embryos		
IVF — oocytes donation#		
Immunosuppressants		
Antiepileptics		

— factors currently included in the risk calculation of trisomy 21 and neural tube defects in the triple test; AFP — alpha-fetoprotein; CVS — chorionic villus sampling; DM — diabetes mellitus; HIV — human immunodeficiency viruses; HSV-2 — herpes simplex virus type 2; ICP — intrahepatic cholestasis of pregnancy; IVF — *in vitro* fertilization; IVF-ICSI — *in vitro* fertilization by intracytoplasmic sperm injection; GDM — gestational diabetes mellitus; PAS — placenta accreta spectrum; PE — preeclampsia; PIH — pregnancy-induced hypertension; PMD — placental mesenchymal dysplasia; OSA — obstructive sleep apnea

black and Caucasian races decreases with increasing gestational age [5–7]. In the first trimester of pregnancy, the difference in AFP levels between black and Caucasian is about 23%, and in the second trimester it is 10–20% [8]. The exact mechanism of these disparities besides the effect of body mass index (BMI) for the yellow race has not yet been determined. The reasons for this are not reported in the literature. Some possible mechanisms, in our opinion, are presented below.

Body mass index

The higher the pre-pregnancy BMI, the lower the maternal serum AFP concentrations. This is due to the dilution

effect in the larger plasma volume [6]. If one were to take this fact into account for Caucasians and correct the AFP results based on the BMI of pregnant women, it would turn out to be at a similar level to Caucasians [5].

Parity

In multiparous women, AFP concentrations are lower than in primiparous women of the same gestational age. The explanation for this phenomenon is believed to be the relationship between synthesis of AFP and estriol. In animal experiments, estriol has been shown to be responsible for AFP synthesis. With successive pregnancies, estriol levels decrease and AFP levels decrease in parallel [9]. AFP levels are related to the length of the interval between successive pregnancies (it is lower the shorter the interval) and the weight of the newborn from the previous birth [10].

Gestational age

AFP levels increase as pregnancy progresses by 37% per week [8]. In the context of underestimation of AFP results expressed in MoM, attention should be paid to redating the gestational age in case of discrepancies between last menstrual period (LMP) and first trimester ultrasound. This would improve the accuracy of calculating AFP MoM values [11]. The cases of amenorrhea, where the crown rump length (CRL) measurement will be underestimated on average by a value equivalent to two days, will be problematic [12].

Twin pregnancies

In twin pregnancies, AFP levels are doubled as are inhibin-A levels, which is explained by the existence of a dual source in the form of two fetuses, while beta-hCG and estriol are not doubled. The results of the research showed that chorionicity has no influence on the AFP level in the maternal serum. Neither does maternal age nor maternal weight. There is a scarcity of randomized controlled trials about marker levels in twin pregnancies in situations with one affected fetus. Besides, even assuming that the fetuses are of equal weight, it is not possible to fully investigate the exact contribution of each fetus to the total measured AFP level. The presence of a healthy fetus can disturb the detection of the sick fetus because of averaged results of AFP level. The main problem is the lack of specially dedicated software to calculate the risk for multifetal pregnancies so currently calculators for single pregnancies are used. To calculate the risk of T21 in twin pregnancies in the second trimester one half of a specific marker is used. As a result, false positive and negative results are numerous and the sensitivity of this test for multifetal pregnancies is lower. To avoid this, the separate calculation risk model for twin pregnancy based on the data from local populations should be created but since the NIPT (non-invasive prenatal testing) becomes a more accurate form of the aneuploidy detection the twin calculator based on biochemical markers is not first line goal [13–17].

Cigarettes and alcohol

Cigarettes

In this regard, the results of scientific studies do not agree. Higher levels of MoM AFP by 11% in the 1st trimester and by 2–6% in the 2nd trimester compared to the population of non-smoking women were observed [8, 18–21]. In contrast, a study by Bredaki et al. [10] found an effect of cigarette smoking on AFP levels only in the 1st trimester, no longer in the 2nd and 3rd. Higher AFP levels in the umbilical cord blood of newborns of mothers who smoke cigarettes were also reported, depending on the amount of cigarettes smoked. High levels of AFP in the umbilical cord were inversely correlated with the weight and length of the newborn [22]. Smoking impairs blood flow in the maternal-fetal unit by affecting placental morphology: blocking cytotrophoblast differentiation, decreasing placental villi volume and their invasiveness.

Smoking-induced tissue hypoxia and the presence of methemoglobin and toxins impairs vascularization of placental villi. It probably also affects, in fetal hepatocytes, the expression of genes responsible for post-translational modifications of proteins responsible for detoxification and secretion, among others [22].

Alcohol

There is little data on the effect of alcoholism on AFP levels. High levels of AFP are found in the serum of chronic and heavy drinking mothers, this happens in the mechanism of fetal liver damage [23].

Fetal weight from a previous birth

No correlation was found between neonatal weight and maternal serum AFP levels in physiological pregnancy. In newborns with low birth weight, elevated AFP levels in pregnant women's serum are related to associated pregnancy pathology. High AFP levels are the result of damage to the placenta and an increase in its permeability such as in preeclampsia and are not a factor per se due to fetal weight [7].

Gender of fetus

Female fetuses have slightly lower AFP values (about 5%) than male fetuses, regardless of racial origin, but in turn are found to have higher levels of beta-hCG [23]. It was suspected that this might result in more frequent false-positive triple test results in mothers of girls and false-negative results in mothers of boys. It turned out that in contrast to pregnancies with genetically normal fetuses, no statistically significant relationship was found between fetal sex and

maternal serum AFP and beta-hCG levels in pregnancies with T21 [24]. There are also studies in which the relationship between AFP and gender was not confirmed [7]. Lower AFP values in female fetuses have been associated with lower fetal liver weight, which was not confirmed in studies on terminated fetuses. Another explanation for the higher AFP production in male fetuses is that AFP plays a protective role against brain exposure to circulating maternal estrogen (it is a carrier protein for it), preventing its feminization [9].

INTERNAL DISEASES

Diabetes mellitus (DM) types 1 and 2

Both groups have lower serum AFP MoM values than women without diabetes. Abnormalities in the development and function of fetal hepatocytes of mothers with insulin-dependent diabetes and immaturity of placental villi in insulin-dependent and independent diabetes have been cited as potential reasons [25–27]. In the 1990s, a 20% correction of AFP MoM levels for diabetes alone was applied. Nowadays, it is believed that only the correction related to the inclusion of BMI is sufficient. Without weight-related correction of AFP MoM, patients with DM 2 have lower values than those with DM 1 because they often have higher body weight. In contrast, after correction using maternal weight, pregnant women with both types of DM have similar levels, but still lower than healthy patients [25]. There is a hypothesis that this approximately 20% lower AFP MoM level is transformed alpha-fetoprotein (tAFP) arising from chronic fetal stress.

The relationship between AFP levels and glycated hemoglobin (HbA1c) levels was also studied. Early reports suggested that patients with poorly controlled diabetes (high HbA1c) had low AFP levels, but this was later not confirmed. On the other hand, most of the studies on the relationship between AFP and diabetes date from the 1980s and 1990s, and given the now much better glycemic control, use of insulins and surveillance of pregnant women with diabetes, there is a need for more recent data. Adequate assessment of AFP levels in these patients is important because the incidence of neural tube defects in children of patients with insulin-dependent diabetes is 3–4 times higher [6].

Chronic hypertension

There was no effect on AFP levels, even in renal transplant patients or those with end-stage renal failure, where kidney function is significantly altered [28].

Systemic lupus erythematosus (SLE)

Pregnant patients with lupus have higher AFP levels. This may be related to the use of glucocorticosteroids (see impact of medicines and supplements) [29]. The second theory is that the underlying cause of high AFP is increased permeability of placental vessels associated with their inflammation in SLE [30].

Thrombophilia

The effect of thrombophilia on AFP levels is still unclear. Congenital thrombophilia either showed no difference in AFP levels compared to controls [31, 32] or showed reduced levels [33] or elevated levels [31]. Some authors contribute that some patients with high AFP levels are carriers of the C677T methylenetetrahydrofolate reductase (MTHFR) mutation [34]. In antiphospholipid syndrome (acquired thrombophilia), elevated AFP levels are noted [35]. The immunosuppressive effect of high AFP concentrations helps reduce the level of antibodies to b2-glycoprotein in antiphospholipid syndrome [32, 31]. It is likely that in addition to increased synthesis as a target for the immunosuppressive effect, elevated maternal serum AFP levels result from increased leakage through a functionally or structurally abnormal placenta. Microscopically, the placentas of patients with thrombophilia may show acquired intervillous thrombosis, chronic chorioamnionitis and placental vascular infarction [36].

There has also been no evidence of any effect of heparins used to treat congenital and acquired thrombophilia on AFP levels, although data on the timing of treatment with these drugs is also often lacking [33].

Obstructive sleep apnea (OSA)

Obstructive sleep apnea (OSA) mainly affects obese pregnant women with an initial physiologically increased respiratory effort. Episodes of apnea cause stimulation of the sympathetic nervous system with concomitant hypoperfusion at the tissue level up to ischemia, and consequently tissue hypoxia and oxidative stress. Clinically, OSA is associated with obstetric complications like hypertension and FGR, among others. High levels of markers of chronic hypoxia: erythropoietin, IL-6 and nuclear forms of red blood cells have been found in the cord blood of newborns of mothers suffering from OSA. When studying the relationship between AFP levels and OSA, it was expected to be high due to ischemic placental damage. Ultimately, however, after using weight correction in calculating the AFP MoM (OSA patients generally have higher BMIs), no statistically significant difference was shown [37].

PATHOLOGICAL CONDITIONS OF PREGNANCY

Gestational diabetes mellitus (GDM)

GDM affects 1–3% of pregnant women. These patients have lower AFP values, and weight correction for AFP MoM is required. There are reports that very low AFP levels are associated with high birth weight of newborns [38].

Hypertension in pregnancy (PIH) and preeclampsia (PE)

Elevated AFP is not found in pregnancy-induced hypertension, but in preeclampsia it is. The greater the severity of preeclampsia, the higher the AFP concentrations in the pregnant woman. This is a consequence of progressively more severe damage to the placental fetal-maternal barrier and increased permeability to AFP. Abnormal placentation and the subsequent maternal inflammatory response (overproduction of anti-angiogenic factors) are the basis for this pathology [30, 38, 39].

Intra- hepatic cholestasis of pregnancy (ICP)

The effect of maternal cholestasis on the liver and biliary functions of the newborn has been demonstrated in an animal model. In the fetus, utilization of bile acids is impossible due to the immaturity of the liver to secrete them and the small intestine to absorb them, and accumulation in the fetus would be toxic. Typically, bile acids from the fetus are transported across the placenta to the mother, where they are excreted into bile in the case of a properly functioning maternal liver [40]. Pregnancy cholestasis impairs maternal and indirectly fetal excretion of bile acids, leads to their accumulation in the fetal liver and impairs its metabolism. However, human studies have not confirmed the effect of this disease on maternal serum AFP levels [41, 42].

Hyperemesis gravidarum

They occur in the first and early second trimester of pregnancy and affect about 3% of pregnant women. No effect on maternal serum AFP levels has been demonstrated [43].

Maternal anemia

Placental volume gain in the second trimester is inversely proportional to maternal hemoglobin levels. In cases of high maternal hemoglobin and accompanying reduced placental volume and dimensions, fetal-maternal AFP transport decreases. It was therefore expected that in cases of maternal anemia, increased placental volume would result in increased transport of AFP to the mother and elevated AFP concentrations in her blood. However, contrary to assumptions, maternal anemia was found to result in low AFP levels. This is attempted to be explained by a dilution of AFP concentrations due to an increase in plasma volume, but on the other hand, high AFP levels should be found in patients with polycythemia and hemoconcentration, and this is not the case [44].

Serological conflict

In the 1970s, the use of AFP was proposed to monitor the course of serological conflict, as its levels were increased in maternal serum and amniotic fluid during this condition. However, due to the development of ultrasonography and Doppler techniques (middle cerebral artery peak systolic velocity — MCA-PSV evaluation), the use of AFP was discontinued.

Two theories attempt to explain the increased serum AFP levels in Rh-negative pregnant women. The first (more likely) speaks of increased AFP production as a side effect of increased hematopoiesis involving a functionally immature fetal liver. The other says there is increased transfer of AFP through a large and swollen placenta, but this does not explain the high levels in amniotic fluid. It seems that increased AFP production precedes the onset of fetal anemia, as an expression of effective adaptation to hemolysis. The more advanced the hemolytic anemia and increasing fetal edema, the paradoxically lower the level of AFP in the mother's blood, due to the breakdown of hepatic hematopoiesis [45].

ASSISTED REPRODUCTION METHODS

In vitro fertilization (IVF)

It has been studied that the FPR in the triple test for assisted reproduction methods is 19%, and the highest at 30.8% in the group using frozen embryos [46]. Pregnancies obtained from IVF have about 10% higher AFP levels in the first trimester of pregnancy compared to those conceived naturally [8]. It seems a fair observation that the number of embryos transferred into the uterus may affect the results of biochemical tests [47]. On the other hand, in the second trimester, pregnancies from IVF have AFP levels about 5% lower than those conceived naturally — hence the higher risk of false positive results in the triple test. Pregnancies from IVF-ICSI (in vitro fertilization by intracytoplasmic sperm injection) have lower AFP MoM than those from conventional IVF (by 16%) and natural conception (by 19%) [48].

Frozen embryos

Patients in IVF pregnancies from frozen embryos have higher serum AFP levels compared to natural conceptions. This is especially true for frozen embryos obtained by conventional IVF (by 20%); frozen embryos obtained by ICSI have standard levels as from natural conceptions. This is influenced by the freezing and thawing process [46, 48, 49].

Oocyte donation

In these pregnancies, AFP levels are higher compared to pregnancies obtained from own oocytes (IVF and natural conception) regardless of the age of the oocyte donor or recipient. This is likely due to impaired implantation, immunoregulation and subsequent vascular disorders in the maternal-fetal unit of oocyte donor pregnancies, resulting in pregnancy pathologies such as pre-eclampsia (PE) or fetal growth restriction (FGR) [47, 50].

Vanishing twin syndrome

When one of the twins dies and is absorbed in the first trimester of pregnancy, AFP levels are elevated by about 10%. The death of one of the twins before the date of prenatal testing can affect the results of biochemical tests of the remaining single fetus. Data obtained over pregnancies after selective termination of one of the fetuses in a twin pregnancy show that AFP levels are elevated for about eight weeks after the procedure. In assessing the genetic risk in this case, two facts would have to be considered. The first is that AFP levels undergo a slow gradual decrease from the time of termination until AFP is measured, and the second is that the half-life of AFP in maternal serum is 59–133 h [51].

DISORDERS OF THE CHORION, PLACENTA AND UTERUS

Chorionic villus sampling (CVS)

Patients after chorionic villus biopsy, as well as any other genetic invasive procedure, show an increase in serum AFP levels, which occurs immediately after the procedure. Maximum values are noted about 1 h after the procedure, after which the level stabilizes. The increase in AFP levels is due to damage to the maternal-fetal barrier. Based on the rise in AFP after the procedure, it is possible to estimate the amount of fetal blood that has entered the mother's bloodstream. This method is comparable to the Kleihauer-Betke test (98% vs 100%). There is a correlation between the weight of the collected villi and the AFP increase. It has not been reported that high AFP values after chorionic villi biopsy persist until 16–18 week of pregnancy, i.e., the standard period for performing a triple or quadruple test [52, 53].

Subchorionic hematoma

In about 20% of women, bleeding in the first trimester of pregnancy is associated with a subchorionic hematoma. The presence of hematoma is associated with an increased risk of miscarriage, preterm labor and FGR. Elevated AFP levels in these patients are due to extravasation of blood into the space between the chorionic villi and the uterine wall, which allows a certain amount of AFP to enter the mother's serum [54].

Placental mesenchymal dysplasia (PMD)

It is a rare vascular pathology of the placenta resulting in placentomegaly with multiple cystic areas preceded by hypoechoic areas. The etiology is unknown. Histopathologically, aneurysmal dilatation of chorionic vasculature with villous proliferation is described, without trophoblastic proliferation. It is often mistaken on ultrasound and histopathological examination for a hydatidiform mole (due to numerous small cystic lesions), in contrast to which there is no risk of transformation into gestational trophoblastic disease and fetal development is usually normal. A high AFP level is accompanied by a normal beta-HCG level for a given gestational age and a normal fetal karyotype (helps distinguish it from hydatidiform mole). High AFP levels are due to the large surface area of the placenta made up of thin-walled vessels, which increases fetal-maternal transfer of AFP. More than 90% of pregnancies with PMD are characterized by complications including pregnancy-induced hypertension (PIH), preeclampsia (PE), hemolysis, elevated liver enzymes, low platelets (HELLP), fetal growth restriction (FGR), fetal anemia, thrombocytopenia, intrauterine fetal demise (IUFD) and preterm birth (PTB) [55].

Placenta accreta spectrum (PAS)

Under normal conditions, the basal decidua is a barrier to the ingrowth of placental villi into the myometrium. If there is even the slightest damage to the basal decidua (e.g., by cesarean section, curettage, endometrial ablations), the decidualization process is disrupted and invasion into the myometrium occurs. It has been studied that the endometrium within the lower uterine segment is less well nourished. Hence, in the case of implantation in this area of the uterus, further damaged by surgery, there is a greater risk of placenta ingrowth. In addition, abnormal placentation is accompanied by pathologically enhanced angiogenesis and hyperperfusion, which increases the absorption of AFP and beta-hCG into the maternal circulation [56].

Circumvallate placenta

Is a pathology of the shape of the placenta. It arises when the chorionic plate in the placenta is smaller than the basal plate, resulting in the formation of a characteristic ring at the periphery on the fetal side. The ring that forms the bulwark is made up of two layers of amniotic fluid and chorionic villi with a layer of degenerated decidua between them and the presence of hematomas and areas of infarction. One theory of this abnormality origin is that the blastocyst implants too deeply into the uterine muscle. It can result in recurrent bleeding from the first trimester of pregnancy, thrombocytopenia, premature rupture of the amniotic membranes, premature separation of the placenta, preterm labor, or fetal growth restriction. The elevated AFP levels found in this case are due to hypoperfusion of the placental plate and the presence of infarcted areas [57].

Uterine myomas

They account for 95% of benign uterine tumors. They occur in 30% of Caucasian women over the age of 35, and an increasing number of pregnant women are now being reported in this age group. Co-occurrence of uterine myomas with pregnancy occurs in about 1–4%. No effect has been shown on AFP levels in pregnancy [58].

INFECTIONS

Human immunodeficiency virus (HIV)

About 90% of HIV-infected women are of reproductive age. Previous scientific studies have shown that there is an effect of HIV infection and the drugs used in the infection on the results of biochemical screening tests, although there are no studies that provide answers as to how HIV affects trophoblast cells [9]. High AFP levels correlate with high viral load and low CD4+ levels, which in this situation increases the risk of a false-negative result in the triple test. HIV+ pregnant women who are untreated have lower AFP levels than cART-treated and healthy women [59]. This is explained by the impaired effect of the virus on placental transport of AFP. Treatment with the combination antiretroviral therapy (cART) regimen increases AFP levels, but they remain within normal limits [60]. These are important data because of the risk of viral transmission during amniocentesis [9].

Intrauterine herpes simplex virus type 2 (HSV-2) infection

It is accompanied by changes in ultrasound images: hyperechoic intestines, hyperechoic foci in the myocardium, differentiated echogenicity of the fetal liver. The source of high AFP can be found in the damage to the fetal liver by the herpes virus in an already advanced infection. High levels of AFP in maternal serum and amniotic fluid in the second trimester are accompanied by high levels of acetylcholesterase in the amniotic fluid (which is typical of open neural tube defects) but in this disease is associated with fetal central nervous system (CNS) damage and necrosis [61].

Parvovirus B19 (B19V)

Parvovirus B19 infects rapidly dividing cells, and this is what attempts to explain its preference for the fetus. Typically, B19V attacks precursor cells of the erythroid lineage in the bone marrow and liver of the fetus leading to complete blockade of erythrocyte production and anemia. The target receptor for B19V is erythrocyte P antigen, which is also found on megakaryocytes, endothelial cells, in the fetal liver and heart. High AFP levels occur based on fetal liver damage and increased transudate through the placenta, which is swollen due to anemia [30].

MEDICINES AND SUPPLEMENTS

The following drugs were not found to affect AFP levels: heparins, antibiotics, painkillers, antidepressants, antiemetics, anti-asthmatics, hypotensives [62, 63].

Antiepileptic drugs such as lamotrigine, levetiracetam, carbamazepine, oxcarbamazepine, valproic acid and clonazepam affect the increase in AFP and E3 levels in the second trimester of pregnancy [64].

Immunosuppressants, particularly corticosteroids, cause elevated AFP levels based on effects on metabolic pathways. Corticosteroids are utilized in the liver involving cytochrome P450, and induction of this group of enzymes has a parallel effect on increasing AFP synthesis. In addition, corticosteroids affect placental perfusion, thereby increasing fetal-maternal transfer of AFP [62].

Protease inhibitors used to treat HIV infection cause AFP levels to decrease. This is important because of the risk of exposing HIV+ pregnant patients to invasive testing for in-depth diagnosis of trisomy 21 in the case of an abnormal triple test [62].

Drugs for ovulation induction: higher AFP levels are found with clomiphene citrate (Clostilbegyt) and lower with gonadotropins, e.g., Pergonal, Menopur, Mensinorm [8].

Folic acid: when food fortification with folic acid began in the US in 1998, it helped reduce the number of patients with AFP levels > 3 MoM by more than 42% [65].

STATISTICAL ERRORS IN RISK ESTIMATION

The basis for estimating the risk of T21 in biochemical tests is the calculation of multiples of the median (MoM) for each component of the test. This allowed us to obtain values comparable for laboratories using the same immunoassay. The calculated MoM value is expected to be corrected for gestational age, patient weight, race, smoking, comorbidities, and periodically monitored to see if it is near 1. Errors in the calculation of the MoM can occur in a number of ways including incorrect calculation of medians (medians must be representative of the population), changes in reagent lot quality, immunoenzymatic assay performance, and human error. Accurate determination of gestational age and reliable regression coefficients for the relationship between biochemical test results and gestational age are also important. Problems with immunoenzymatic assay and incorrectly determined regression equations can result in deviations in the determination of MoM values. It has been found that a 10% error in calculating MoM values for a single marker (which is reportedly quite common) can result in a 1–2% increase in FPR in a triple test. If it involves all three markers, then the FPR can even double [66].

CONCLUSIONS

1. The AFP level, like other biochemical markers, should not be analyzed only as the current serum concentration without considering the influence of demographic factors, ongoing diseases, medications used, type of pregnancy, pathological conditions and abnormalities of pregnancy and other disturbances that imply an appropriate correction of the concentration expressed by MoM to increase the accuracy of prenatal testing.

2. The impact of statistical errors in estimating the risk of T21 and neural tube defects is little appreciated. Failure to account for the above factors increases false-positive high-risk results and following unnecessary invasive testing for T21.
3. AFP, due to a number of correlations with disease states in pregnancy, shows potential as a more universal marker of fetal and maternal well-being.
4. Patients should be informed of the sensitivity and limitations of each screening test to avoid unnecessary stressful situations.
5. There is a lack of research on the effects of various factors on AFP levels in the first and third trimesters of pregnancy.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Conde-Agudelo A, Kafury-Goeta AC. Triple-marker test as screening for Down syndrome: a meta-analysis. Obstet Gynecol Surv. 1998; 53(6): 369–376, doi: 10.1097/00006254-199806000-00022, indexed in Pubmed: 9618713.
2. Wald NJ, Hackshaw AK. Advances in antenatal screening for Down syndrome. Baillieres Best Pract Res Clin Obstet Gynaecol. 2000; 14(4): 563–580, doi: 10.1053/beog.2000.0097, indexed in Pubmed: 10985931.
3. Wald NJ, Rodeck C, Hackshaw AK, et al. SURUSS in Perspective. Semin Perinatol. 2005; 29(4): 225–235, doi: 10.1053/j.semperi.2005.05.006, indexed in Pubmed: 16104673.
4. Glowska-Ciemny J, Pankiewicz J, Malewski Z, et al. Alpha-fetoprotein (AFP) - new aspects of a well-known marker in perinatology. Ginekol Pol. 2022 [Epub ahead of print], doi: 10.5603/GP.a2021.0226, indexed in Pubmed: 35072257.
5. O'Brien JE, Dvorin E, Drugan A, et al. Race-ethnicity-specific variation in multiple-marker biochemical screening: alpha-fetoprotein, hCG, and estriol. Obstet Gynecol. 1997; 89(3): 355–358, doi: 10.1016/S0029-7844(96)00524-8, indexed in Pubmed: 9052584.
6. Krantz DA, Hallahan TW, Carmichael JB. Screening for open neural tube defects. Clin Lab Med. 2016; 36(2): 401–406, doi: 10.1016/j.cll.2016.01.004, indexed in Pubmed: 27235920.
7. Lagiou P, Samoli E, Lagiou A, et al. Levels and correlates of alpha-fetoprotein in normal pregnancies among Caucasian and Chinese women. Eur J Cancer Prev. 2007; 16(3): 178–183, doi: 10.1097/01.cej.0000228414.00634.53, indexed in Pubmed: 17415087.
8. Bredaki FE, Wright D, Akolekar R, et al. Maternal serum alpha-fetoprotein in normal pregnancy at 11-13 weeks' gestation. Fetal Diagn Ther. 2011; 30(4): 274–279, doi: 10.1159/000330200, indexed in Pubmed: 22156386.
9. Brossard P, Boulvain M, Coll O, et al. Swiss HIV Cohort Study, Swiss HIV Mother and Child Cohort Study. Is screening for fetal anomalies reliable in hiv-infected pregnant women? A multicentre study. AIDS. 2008; 22(15): 2013–2017, doi: 10.1097/QAD.0b013e32830fbda3, indexed in Pubmed: 18784463.
10. Bredaki FE, Sciorio C, Wright A, et al. Serum alpha-fetoprotein in the three trimesters of pregnancy: effects of maternal characteristics and medical history. Ultrasound Obstet Gynecol. 2015; 46(1): 34–41, doi: 10.1002/uog.14809, indexed in Pubmed: 25652769.
11. Dashe JS, Twickler DM, Santos-Ramos R, et al. Alpha-fetoprotein detection of neural tube defects and the impact of standard ultrasound. Am J Obstet Gynecol. 2006; 195(6): 1623–1628, doi: 10.1016/j.ajog.2006.03.097, indexed in Pubmed: 16769022.
12. Bredaki FE, Poon LC, Birdir C, et al. First-trimester screening for neural tube defects using alpha-fetoprotein. Fetal Diagn Ther. 2012; 31(2): 109–114, doi: 10.1159/000335677, indexed in Pubmed: 22377693.
13. Ren F, Hu YU, Zhou H, et al. Second trimester maternal serum triple screening marker levels in normal twin and singleton pregnancies. Biomed Rep. 2016; 4(4): 475–478, doi: 10.3892/br.2016.611, indexed in Pubmed: 27073636.
14. O'Brien J, Dvorin E, Yaron Y, et al. Differential increases in AFP, hCG, and uE3 in twin pregnancies: Impact on attempts to quantify Down syndrome screening calculations. Am J Med Genet. 1997; 73(2): 109–112, doi: 10.1002/(sici)1096-8628(19971212)73:2<109::aid-ajmg1>3.0.co;2-p, indexed in Pubmed: 9409857.
15. Watt HC, Wald NJ, Georg L. Maternal serum inhibin-A levels in twin pregnancies: implications for screening for Down's syndrome. Prenat Diagn. 1996; 16(10): 927–929, doi: 10.1002/(sici)1097-0223(199610)16:10<927::aid-pd972>3.0.co;2-0, indexed in Pubmed: 8938062.
16. Muller F, Dreux S, Dupoizat H, et al. Second-trimester Down syndrome maternal serum screening in twin pregnancies: impact of chorionicity. Prenat Diagn. 2003; 23(4): 331–335, doi: 10.1002/pd.594, indexed in Pubmed: 12673641.
17. Chen Y, Yang F, Shang X, et al. A study on non-invasive prenatal screening for the detection of aneuploidy. Ginekol Pol. 2022; 93(9): 716–720, doi: 10.5603/GP.a2021.0254, indexed in Pubmed: 35315016.
18. Urato AC, Quinn R, Pulkkinen A, et al. Maternal smoking during pregnancy and false positive maternal serum alpha-fetoprotein (MSAFP) screening for open neural tube defects. Prenat Diagn. 2008; 28(8): 778–780, doi: 10.1002/pd.2029, indexed in Pubmed: 18551721.
19. Crossley JA, Aitken DA, Waugh SML, et al. Maternal smoking: age distribution, levels of alpha-fetoprotein and human chorionic gonadotrophin, and effect on detection of Down syndrome pregnancies in second-trimester screening. Prenat Diagn. 2002; 22(3): 247–255, doi: 10.1002/pd.313, indexed in Pubmed: 11920904.
20. Beratis NG, Varvarigou A, Christophidou M, et al. Cord blood alpha-fetoprotein concentrations in term newborns of smoking mothers. Eur J Pediatr. 1999; 158(7): 583–588, doi: 10.1007/s004310051152, indexed in Pubmed: 10412820.
21. Rudnicka AR, Wald NJ, Huttly W, et al. Influence of maternal smoking on the birth prevalence of Down syndrome and on second trimester screening performance. Prenat Diagn. 2002; 22(10): 893–897, doi: 10.1002/pd.440, indexed in Pubmed: 12378572.
22. Odendaal HJ. Effects of alcohol, cigarettes, methamphetamine and marijuana exposure during pregnancy on maternal serum alpha-fetoprotein levels at 20-24 weeks'gestation. J Pediatr Neonatal Care. 2018; 8(1): :00314. Epub 2018, doi: 10.15406/jpnc.2018.08.00314, indexed in Pubmed: 31106259.
23. Mueller VM, Huang T, Summers AM, et al. The effect of fetal gender on the false-positive rate of Down syndrome by maternal serum screening. Prenat Diagn. 2005; 25(13): 1258–1261, doi: 10.1002/pd.1318, indexed in Pubmed: 16353275.
24. Lehavi O, Aizenstein O, Evans MI, et al. 2nd-trimester maternal serum human chorionic gonadotropin and alpha-fetoprotein levels in male and female fetuses with Down syndrome. Fetal Diagn Ther. 2005; 20(3): 235–238, doi: 10.1159/000083911, indexed in Pubmed: 15824504.
25. Thornburg LL, Knight KM, Peterson CJ, et al. Maternal serum alpha-fetoprotein values in type 1 and type 2 diabetic patients. Am J Obstet Gynecol. 2008; 199(2): 135.e1–135.e5, doi: 10.1016/j.ajog.2008.02.046, indexed in Pubmed: 18455133.
26. Sancken U, Bartels I. Biochemical screening for chromosomal disorders and neural tube defects (NTD): is adjustment of maternal alpha-fetoprotein (AFP) still appropriate in insulin-dependent diabetes mellitus (IDDM)? Prenat Diagn. 2001; 21(5): 383–386, doi: 10.1002/pd.72, indexed in Pubmed: 11360279.
27. Huttly W, Rudnicka A, Wald NJ. Second-trimester prenatal screening markers for Down syndrome in women with insulin-dependent diabetes mellitus. Prenat Diagn. 2004; 24(10): 804–807, doi: 10.1002/pd.994, indexed in Pubmed: 15503275.
28. Shenhav S, Gemer O, Sherman DJ, et al. Midtrimester triple-test levels in women with chronic hypertension and altered renal function. Prenat Diagn. 2003; 23(2): 166–167, doi: 10.1002/pd.564, indexed in Pubmed: 12575027.
29. La Montagna G, Baruffo A, Buono G, et al. False positivity of prenatal Down's syndrome and neural-tube tests in SLE. Lancet. 2000; 356(9236): 1194–1195, doi: 10.1016/S0140-6736(05)72892-0, indexed in Pubmed: 11030323.
30. Mizejewski GJ. Levels of alpha-fetoprotein during pregnancy and early infancy in normal and disease states. Obstet Gynecol Surv. 2003; 58(12): 804–826, doi: 10.1097/01.OGX.0000099770.97668.18, indexed in Pubmed: 14668662.
31. Frank M, Maymon R, Wiener Y, et al. The effect of hereditary versus acquired thrombophilia on triple test Down's syndrome screening.

Prenat Diagn. 2013; 33(2): 191–195, doi: 10.1002/pd.4041, indexed in Pubmed: 23292892.
32. Fialová L, Malhoban I, Mikulíková L, et al. Antibodies against beta 2-glycoprotein I (beta 2-GP I) and their relationship to fetoplacental antigens. Physiol Res. 2002; 51(5): 449–455, indexed in Pubmed: 12470197.
33. Derbent AU, Yanik FF, Inegöl Gümüş I, et al. Impact of inherited thrombophilias on first and second trimester maternal serum markers for aneuploidy. J Matern Fetal Neonatal Med. 2012; 25(11): 2177–2181, doi: 10.3109/14767058.2012.684105, indexed in Pubmed: 22506595.
34. Björklund NK, Evans JA, Greenberg CR, et al. The C677T methylenetetrahydrofolate reductase variant and third trimester obstetrical complications in women with unexplained elevations of maternal serum alpha-fetoprotein. Reprod Biol Endocrinol. 2004; 2: 65, doi: 10.1186/1477-7827-2-65, indexed in Pubmed: 15352998.
35. Hong SY, Kim SY, Kim JH, et al. Predictive value of quad serum markers for adverse pregnancy outcome in antiphospholipid antibody syndrome. Lupus. 2021; 30(6): 981–990, doi: 10.1177/0961203211001126, indexed in Pubmed: 33709835.
36. Ochshorn Y, Kupferminc MJ, Eldor A, et al. Second-trimester maternal serum alpha-fetoprotein (MSAFP) is elevated in women with adverse pregnancy outcome associated with inherited thrombophilias. Prenat Diagn. 2001; 21(8): 658–661, doi: 10.1002/pd.119, indexed in Pubmed: 11536265.
37. Salameh M, Lee J, Palomaki G, et al. Snoring and markers of fetal and placental wellbeing. Clin Chim Acta. 2018; 485: 139–143, doi: 10.1016/j.cca.2018.06.039, indexed in Pubmed: 29958890.
38. Räty R, Anttila L, Virtanen A, et al. Maternal midtrimester free beta-HCG and AFP serum levels in spontaneous singleton pregnancies complicated by gestational diabetes mellitus, pregnancy-induced hypertension or obstetric cholestasis. Prenat Diagn. 2003; 23(13): 1045–1048, doi: 10.1002/pd.751, indexed in Pubmed: 14691989.
39. Rana S, Lemoine E, Granger JP, et al. Preeclampsia: pathophysiology, challenges, and perspectives. Circ Res. 2019; 124(7): 1094–1112 [Erratum in: Circ Res. 2020 Jan 3;126(1):e8.], doi: 10.1161/CIRCRESAHA.118.313276, indexed in Pubmed: 30920918.
40. Türkmen GG, Timur H, Yilmaz Z, et al. Effect of intrahepatic cholestasis of pregnancy on maternal serum screening tests. J Neonatal Perinatal Med. 2016; 9(4): 411–415, doi: 10.3233/NPM-161618, indexed in Pubmed: 28009332.
41. Monte MJ, Morales AI, Arevalo M, et al. Reversible impairment of neonatal hepatobiliary function by maternal cholestasis. Hepatology. 1996; 23(5): 1208–1217, doi: 10.1002/hep.510230540, indexed in Pubmed: 8621155.
42. Tayyar AT, Tayyar A, Atakul T, et al. Could first- and second-trimester biochemical markers for Down syndrome have a role in predicting intrahepatic cholestasis of pregnancy? Arch Med Sci. 2018; 14(4): 846–850, doi: 10.5114/aoms.2017.69865, indexed in Pubmed: 30002703.
43. Peled Y, Melamed N, Krissi H, et al. The impact of severe hyperemesis gravidarum on the triple test screening results. J Matern Fetal Neonatal Med. 2012; 25(6): 637–638, doi: 10.3109/14767058.2011.594468, indexed in Pubmed: 21819342.
44. Bartha JL, Omar KM, Soothill PW. The relationship between maternal serum alpha-fetoprotein and maternal haemoglobin. Prenat Diagn. 2004; 24(8): 609–612, doi: 10.1002/pd.921, indexed in Pubmed: 15305347.
45. Bartha JL, Harrison G, Kyle PM, et al. Fetal serum alpha-fetoprotein in alloimmunised pregnancies. Prenat Diagn. 2003; 23(11): 917–920, doi: 10.1002/pd.728, indexed in Pubmed: 14634978.
46. Räty R, Virtanen A, Koskinen P, et al. Serum free beta-HCG and alpha-fetoprotein levels in IVF, ICSI and frozen embryo transfer pregnancies in maternal mid-trimester serum screening for Down's syndrome. Hum Reprod. 2002; 17(2): 481–484, doi: 10.1093/humrep/17.2.481, indexed in Pubmed: 11821299.
47. Gupta S, Fox NS, Rebarber A, et al. Biochemical screening for aneuploidy in patients with donor oocyte pregnancies compared with autologous pregnancies. J Matern Fetal Neonatal Med. 2014; 27(14): 1418–1421, doi: 10.3109/14767058.2013.866644, indexed in Pubmed: 24228730.
48. Lam YH, Yeung WS, Tang MH, et al. Maternal serum alpha-fetoprotein and human chorionic gonadotrophin in pregnancies conceived after intracytoplasmic sperm injection and conventional in-vitro fertilization. Hum Reprod. 1999; 14(8): 2120–2123, doi: 10.1093/humrep/14.8.2120, indexed in Pubmed: 10438437.
49. Hui PW, Lam YH, Tang MH, et al. Maternal serum hCG and alpha-fetoprotein levels in pregnancies conceived after IVF or ICSI with fresh and frozen-thawed embryos. Hum Reprod. 2003; 18(3): 572–575, doi: 10.1093/humrep/deg153, indexed in Pubmed: 12615827.
50. Dondik Y, Pagidas K, Eklund E, et al. Levels of angiogenic markers in second-trimester maternal serum from in vitro fertilization pregnancies with oocyte donation. Fertil Steril. 2019; 112(6): 1112–1117, doi: 10.1016/j.fertnstert.2019.07.017, indexed in Pubmed: 31843087.
51. Huang T, Boucher K, Aul R, et al. First and second trimester maternal serum markers in pregnancies with a vanishing twin. Prenat Diagn. 2015; 35(1): 90–96, doi: 10.1002/pd.4492, indexed in Pubmed: 25200921.
52. Katiyar R, Kriplani A, Agarwal N, et al. Detection of fetomaternal hemorrhage following chorionic villus sampling by Kleihauer Betke test and rise in maternal serum alpha feto protein. Prenat Diagn. 2007; 27(2): 139–142, doi: 10.1002/pd.1632, indexed in Pubmed: 17191260.
53. Knott PD, Chan B, Ward RH, et al. Changes in circulating alphafetoprotein and human chorionic gonadotrophin following chorionic villus sampling. Eur J Obstet Gynecol Reprod Biol. 1988; 27(4): 277–281, doi: 10.1016/0028-2243(88)90039-1, indexed in Pubmed: 2454849.
54. Kumbak B, Sahin L. Elevated maternal serum alpha-fetoprotein levels in patients with subchorionic hematoma. J Matern Fetal Neonatal Med. 2010; 23(7): 717–719, doi: 10.3109/14767050903366093, indexed in Pubmed: 19883262.
55. Colpaert RM, Ramseyer AM, Luu T, et al. Diagnosis and management of placental mesenchymal disease. A review of the literature. Obstet Gynecol Surv. 2019; 74(10): 611–622, doi: 10.1097/OGX.0000000000000716, indexed in Pubmed: 31670834.
56. Bartels HC, Postle JD, Downey P, et al. Placenta accreta spectrum: a review of pathology, molecular biology, and biomarkers. Dis Markers. 2018; 2018: 1507674, doi: 10.1155/2018/1507674, indexed in Pubmed: 30057649.
57. Celik OY, Obut M, Keles A, et al. Outcomes of pregnancies diagnosed with circumvallate placenta, and use of uterine artery pulsatility index and maternal serum alpha-fetoprotein for prediction of adverse outcomes. Eur J Obstet Gynecol Reprod Biol. 2021; 262: 57–61, doi: 10.1016/j.ejogrb.2021.05.008, indexed in Pubmed: 33989943.
58. Sieroszewski P, Wierzbicka D, Bober L, et al. Association between uterine leiomyomas and the biochemical screening test results in the first and second trimester of pregnancy: a pilot study. J Matern Fetal Neonatal Med. 2011; 24(7): 904–906, doi: 10.3109/14767058.2010.536864, indexed in Pubmed: 21463216.
59. Le Meaux JP, Tsatsaris V, Schmitz T, et al. Maternal biochemical serum screening for Down syndrome in pregnancy with human immunodeficiency virus infection. Obstet Gynecol. 2008; 112(2 Pt 1): 223–230, doi: 10.1097/AOG.0b013e318180968c, indexed in Pubmed: 18669715.
60. Djeha A, Money D, Trottier H, et al. The association between antiretroviral therapy and early placental function: a cohort study. J Matern Fetal Neonatal Med. 2021; 34(5): 683–689, doi: 10.1080/14767058.2019.1613362, indexed in Pubmed: 31092068.
61. Curtin WM, Menegus MA, Patru MM, et al. Midtrimester fetal herpes simplex-2 diagnosis by serology, culture and quantitative polymerase chain reaction. Fetal Diagn Ther. 2013; 33(2): 133–136, doi: 10.1159/000342756, indexed in Pubmed: 23075531.
62. Pekarek DM, Chapman VR, Neely CL, et al. Medication effects on midtrimester maternal serum screening. Am J Obstet Gynecol. 2009; 201(6): 622.e1–622.e5, doi: 10.1016/j.ajog.2009.07.013, indexed in Pubmed: 19762000.
63. Wiener Y, Frank M, Neeman O, et al. Does low molecular weight heparin influence the triple test result in pregnant women with thrombophilia? Isr Med Assoc J. 2012; 14(4): 247–250, indexed in Pubmed: 22675844.
64. Besimoglu B, Öcal FD, Sinaci S, et al. Effect of antiepileptic drugs on serum biochemical marker levels of first and second trimester screening tests. J Obstet Gynaecol Res. 2022; 48(7): 1607–1612, doi: 10.1111/jog.15250, indexed in Pubmed: 35411996.
65. Evans MI, Llurba E, Landsberger EJ, et al. Impact of folic acid fortification in the United States: markedly diminished high maternal serum alpha-fetoprotein values. Obstet Gynecol. 2004; 103(3): 474–479, doi: 10.1097/01.AOG.0000114984.82549.99, indexed in Pubmed: 14990409.
66. Nix B, Wright D, Baker A. The impact of bias in MoM values on patient risk and screening performance for Down syndrome. Prenat Diagn. 2007; 27(9): 840–845, doi: 10.1002/pd.1791, indexed in Pubmed: 17600858.

VIA MEDICA

CLINICAL VIGNETTE

Ginekologia Polska
2023, vol. 94, no. 2, 167–168

ISSN 0017–0011, e-ISSN 2543–6767

DOI 10.5603/GP.a2022.0159

Metastatic gastric cancer in a full-term pregnancy

Jorge Aparicio-Ponce[1], Sandra Salcedo-Hermoza[2], Sandra Aparicio-Salcedo[3], Gustavo Cerrillo[2, 4], Carlos Nureña[2, 5], Jose S Lazarte[6], Ericson L. Gutierrez[7]

[1]Departamento de Ginecología y Obstetricia, Hospital Nacional Dos de Mayo, Lima, Peru
[2]Facultad de Medicina, Universidad Nacional Mayor de San Marcos, Lima, Peru
[3]Facultad de Medicina, Universidad Ricardo Palma, Lima, Peru
[4]Servicio de Anatomía Patológica, Hospital Nacional Dos de Mayo, Lima, Peru
[5]Servicio de Gastroenterología, Hospital Nacional Dos de Mayo, Lima, Peru
[6]Masterton Medical Centre, 4 Colombo Road, Lansdowne, Masterton 5810, New Zealand
[7]Instituto de Investigaciones en Ciencias Biomédicas (INICIB), Universidad Ricardo Palma, Lima, Peru

Key words: gastric cancer; pregnancy; pregnancy complications; gastric adenocarcinoma; maternal mortality

Ginekologia Polska 2023; 94, 2: 167–168

INTRODUCTION

Gastric cancer is rarely associated with pregnancy. Nevertheless, the incidence has been increasing in recent times [1, 2]. This report aims to increase understanding of the infrequent association between gastric cancer and pregnancy, with the hope that this greater awareness will allow future clinicians to make earlier diagnoses that will lead to better perinatal outcomes.

CASE REPORT

The case is a 24-year-old pregnant woman at 37 weeks of gestation, with an obstetric history of two vaginal births and two abortions. There was no significant medical or family history. The patient was admitted in June 2021, via the Emergency Department, with uterine contractions and mild shortness of breath. She also experienced nausea and vomiting, pelvic pain and difficulty walking for the three weeks prior to admission. On admission, she had a blood pressure of 110/70 mmHg, heart rate of 88 beats per minute, respiratory rate of 22 breaths per minute, temperature of 36.7°C and oxygen saturation of 99%. She had a cervical dilation of 1 cm, 50% effacement, –3 cm foetal station, and intact membranes.

In the context of the COVID-19 pandemic, and due to her respiratory symptoms, an antigen test and a chest X-ray were performed. The results of these were both negative. Other routine laboratory tests were also normal. Given the delay of progression of the first stage of labour, misoprostol was administered to encourage cervical ripening. Subsequently, due to inadequate contractions, endovenous oxytocin was administered. Cervical dilation did not progress beyond 5 cm. Due to arrest of the first stage of labour, a caesarean section was performed.

During the caesarean section, gross inspection of the abdominal cavity showed a thickened nodular parietal peritoneum, although the patient had undergone no previous surgeries. Approximately 3 litres of straw-coloured ascites fluid were also seen. The anterior aspect of the uterus had a pale surface with multiple nodules of miliary appearance. The posterior aspect of the uterus, the fallopian tubes, and the ovaries had a similar appearance. The bladder was collapsed with thickened walls. Multiple yellow nodules were observed in the parietal peritoneum, the surface of the intestines, and on the retroperitoneum, with the largest nodule measuring around 0.5 cm. Bowel walls were also thickened. A general surgeon was called into the operating room to perform peritoneal biopsy samples.

A healthy, 38-week female newborn, appropriate for gestational age, was delivered. She weighed 2,856 grams. She had a favourable clinical course and was discharged on her third day. She had a subsequent clinical examination on the sixth day without any concerns.

Corresponding author:
Jorge Aparicio-Ponce
Departamento de Ginecología y Obstetricia, Hospital Nacional Dos de Mayo, Calle Sor Tita 281, Miraflores, Lima, Peru
phone: +51 963 839 892
e-mail: japariciop@unmsm.edu.pe

Received: 18.08.2022 Accepted: 30.10.2022 Early publication date: 12.01.2023

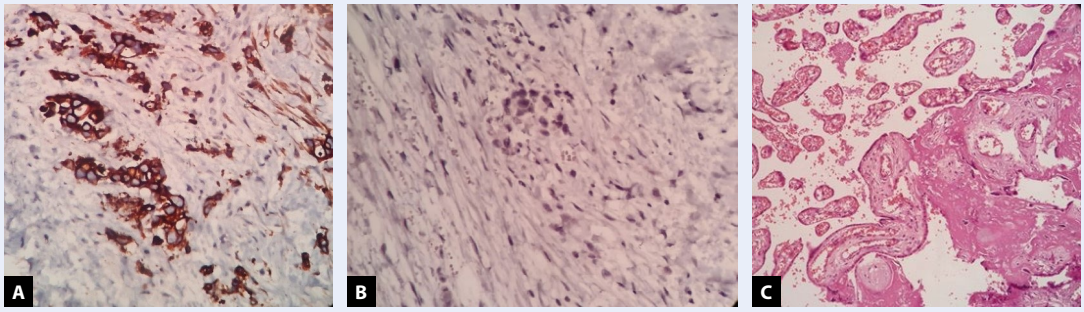


Figure 1. A. Peritoneal biopsy showing poorly differentiated epithelial malignant neoplasia, cytokeratin 7 positive; **B.** Peritoneal biopsy showing poorly differentiated epithelial malignant neoplasia, cytokeratin 20 negative; **C.** Third trimester placenta with intervillous fibrin deposits

Given the regional context, peritoneal tuberculosis was ruled out after the relevant examinations were conducted. The biopsy results showed a poorly differentiated, metastatic epithelial malignancy. Immunohistochemical analysis was positive for cytokeratin 7, and negative for cytokeratin 20. It also showed a focal CDX2 positivity, with negative CDH17 and PAX8 (Fig. 1A, B). Placental histology showed a third-trimester placenta with intervillous fibrin deposits (Fig. 1C).

The patient requested a voluntary discharge against medical advice, on the third day after the caesarean section. She was re-admitted eleven days later, after developing abdominal pain, early satiety and decreased appetite. Ascites was again seen during the physical examination. Subsequent studies were performed to try to identify the primary malignancy. An abdominal CT scan showed nodular thickening of the peritoneum (likely carcinomatosis), thickening of the gastric fundus, inflammatory thickening of the small intestine, as well as moderate fatty changes in the liver. Computed tomography scans of the neck and osseous pelvis were unremarkable. A CT scan of the thorax revealed pulmonary effusions and right lung atelectasis. Tumour marker analysis showed elevated AFP, CA125 and CA199, while CEA and CA153 were within the normal range. An upper gastrointestinal endoscopy was performed, which revealed a Borrmann Type IV gastric neoplasia. Further gastric biopsies showed an infiltrative, poorly differentiated gastric adenocarcinoma. Immunohistochemistry analysis was positive for pan-cytokeratin and negative for CD45.

The patient deteriorated quickly; a nasojejunal transpyloric catheter was placed for enteral feeding. Subsequently, she was transferred to a specialised institution for palliative chemotherapy. She died 35 days post-partum.

CONCLUSIONS

In the literature, 36 weeks was the latest gestational age at which diagnosis for gastric cancer was made, as reported by Yildiz [1] in Turkey. In our case, the delay in diagnosis could have been related to the COVID-19 pandemic. Even though the patient had prenatal follow up, these appointments were primarily held virtually.

The literature also highlights how the frequency of cancer associated with pregnancy is rising, potentially due to increased maternal age. Gastric cancer is no exception. The highest incidence occurs in pregnant individuals between the ages of 30 and 40. These findings are supported by recent reports from Patan [2], Yildiz [1] and Fory [3]. However, in our case, the patient was only 24 years old, similar to the cases reported by Prieto-Montaño [4] and Whittington [5].

In conclusion, given the overlap of symptoms between gastric cancer and pregnancy, a high clinical suspicion is required to make an early diagnosis, which could potentially improve maternal outcomes. The case presented had similar clinical and histologic characteristics as those reported in high incidence regions.

Conflicts of interest

None of the authors reports any conflict of interest.

REFERENCES

1. Yildiz M, Akgun Y, Ozer H, et al. A rare case presentation: pregnancy and gastric carcinoma. BMC Gastroenterol. 2020; 20(1): 33, doi: 10.1186/s12876-020-1184-9, indexed in Pubmed: 32050902.
2. Patan S, Benzar T, Sanford G, et al. Advanced metastatic gastric adenocarcinoma identified within the placenta: a case report with literature review. J Gastrointest Oncol. 2020; 11(1): 127–132, doi: 10.21037/jgo.2019.09.10, indexed in Pubmed: 32175115.
3. A JF, Arenas V, Carmona V, et al. Endocarcinoma gástrico y embarazo reporte de dos casos y revisión de la literatura. Revista Med. 2017; 25(2): 63–73, doi: 10.18359/rmed.3077.
4. Prieto-Montaño J, Reyna-Villasmil E, Rondon-Tapía M. Adenocarcinoma gástrico metastásico en el embarazo. Reporte de caso. Avan Biomed. 2017; 6(1): 70–4.
5. Whittington JR, Whitcombe DD, Eads LE, et al. Signet ring cell carcinoma with lymphangitic carcinomatosis in pregnancy: a case report of an unexpected maternal death and review of the literature. Am J Case Rep. 2019; 20: 1888–1891, doi: 10.12659/AJCR.919412, indexed in Pubmed: 31844036.

V FORUM GINEKOLOGII I PERINATOLOGII PRAKTYCZNEJ

STANDARDY POSTĘPOWANIA W POŁOŻNICTWIE I GINEKOLOGII

Przewodniczący Komitetu Naukowego:
prof. dr hab. n. med. Piotr Sieroszewski

ŁÓDŹ, 8–9 grudnia 2023 roku

Zapraszamy serdecznie na naszego Facebooka!
www.facebook.com/FGiPP

Szczegóły oraz rejestracja na stronie internetowej:

www.forumginekologii.viamedica.pl

Kontakt w sprawie uczestnictwa:

forumginekologii@viamedica.pl

tel.: (58) 320 94 94

PATRONAT

ORGANIZATOR

PATRONAT MEDIALNY

PARTNER

Konferencja jest skierowana tylko do osób uprawnionych do wystawiania recept lub osób prowadzących obrót produktami leczniczymi — podstawa prawna: ustawa z dnia 6 września 2001 r. Prawo farmaceutyczne (t.j. Dz.U. z 2020 r. poz. 944).